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STUDIES ON CRYPTOPLASMIC INFECTION. I, DEVELOPMENT OF A CRYPTOCOCCUS IN CULTURES FROM AN UNCLASSIFIED CHRONIC PHILIPPINE ULCER [1]

By H. W. WADE

(*From the Biological Laboratory, Bureau of Science, Manila*)

FIVE PLATES

GENERAL HYPOTHESIS

In the pathology of fungous infections of the deeper tissues there are sometimes noted features difficult to explain on the basis of the recognized factors of infection. Having been interested by certain such observations on the American "blastomycosis," so-called, I was led to undertake a study of mycotic lesions as they occur in the Philippine Islands. From the outset there were encountered difficulties and peculiarities, and a number of observations have been made that, for their explanation, have led to the formulation of a tentative hypothesis that departs radically from orthodox views. Though it is impossible as yet fully to define these observations, it seems desirable to outline their chief features in order to indicate the line of investigation to which they have led.

Essentially it appears as if the differentiated, demonstrable parasitic element in a mycotic lesion may give rise to a derivative substance, morphologically not differentiated and perhaps even quite amorphous, that, unlike the known "toxins," soluble

[1] This article, though preceded by three others having to do with certain infectious fungi and the lesions caused by them [*Journ. Inf. Dis.* (1916), 18, 618; *Arch. Int. Med.* (1916), 18, 103; and *This Journal, Sec. B* (1916), 11, 267], is the first paper of a contemplated series based on a group of more or less related observations and experimental studies, by the sum total of which it is hoped that the general hypothesis here outlined may be established.

or insoluble, is by itself more or less viable and may be capable, to a certain extent at least and under certain conditions, of persisting as such and even of increasing or growing.

The hypothetical substance or body (to designate which the term "cryptoplasm" seems appropriate), though it may play a more or less important part in the pathogenic expression of the infection, is probably in some cases dependent upon the presence of the original differentiated parasitic element for its continuous replacement and thus for its perpetuation in the lesion, in which case it would be successfully resisted by the host in the absence of the formed element. In other cases, however, it may be able to perpetuate itself and maintain the lesion. Reversion to the differentiated parasitic form may be possible in some cases, under proper conditions; in other cases, and under ordinary conditions, it probably does not occur.

There is evidence that the cryptoplasm is, or may become, intraleucocytic and that, though morphologically indefinable from the protoplasm of the host cell, it may there exert special influences, producing special results, and may sometimes even be indirectly demonstrable therein by cultural methods as a formed, though not necessarily as a specifically differentiated element.

Such a substance, it is evident, might sometimes be of greater immediate importance from the viewpoint of pathogenicity than its formed generator. Should it replace the latter entirely in a progressive lesion, efforts to demonstrate or isolate the causative agent of that lesion by usual methods would of necessity fail. Under such conditions the postulates of Koch would be impossible of fulfillment, since these are based on the essential simplicity of pathogenic organisms and of their modus operandi, on the integrity of form of the invading organism, on its persistence during parasitism and final recoverability in cultures as such, and on the suitability or adaptability of the organism as such to both parasitic and saprophytic life.

The above hypothesis is based on the results of but two years' work and, no doubt, will be more or less modified by future studies. As stated, it has been constructed to cover a group of more or less clear-cut observations. Though much of it cannot as yet be proved, it is what appeals to me as the more conservative of two possible interpretations, the other of which will not be discussed at this point.

INTRODUCTION

In the present report it is desired to describe particularly certain features observed in the course of a cultural study of

material from a series of chronic, apparently mycotic ulcers, in the fresh material from which no parasitic element could be detected. These features have to do with the appearance in the cultures of a differentiated fungous organism, apparently as a result of certain peculiar changes in the very substance of the cells of the infected material.

This is a phenomenon so peculiar, so unlike anything known to have been observed in the study of such infections, and so radically different from the behavior of infectious organisms as generally understood, that it quite overshadows in its possible broader significance whatever interest attaches to the pathologic condition from which the material was obtained. It constitutes an essential contribution to the general hypothesis outlined above. At the present time it is impossible to do more than describe the findings in detail and discuss the apparent explanation. Direct confirmation cannot as yet be had; that must depend upon future studies. Indirect evidence supportive of the explanation suggested has been found in other studies yet to be reported.

Chronic ulcerations of types not definitely classifiable are not uncommon in the Philippine Islands and, apparently, in other parts of the tropics. Many of these are doubtless assignable to syphilis. Chronic ulcers, often associated with destructive lesions, also occur as a late manifestation of yaws in districts where this disease is endemic. There still remain, however, ulcers that are not assignable with probability to any recognized cause; a small group of such lesions is the basis of this report.

Few reports of investigations of such lesions in the Philippine Islands are to be found. Strong [2] described three types of ulcers, in sections from one of which he found bodies that he concluded were fungous. The etiology of the other two remained obscure.

Stitt,[3] before the Sixth International Dermatological Congress (1907), said that failing to find tropical ulcer as described in the textbooks he had concluded that it did not occur in the Philippines, though ulcers due to neglected wounds and to the infective granulomata were common. In Guam, also, none were found, though extensive and mutilating ulcerations were frequently seen. Among Americans, for the most part sailors in the Naval Hospital at Cañacao, Philippine Islands, he observed two distinct types. One began as a painless swelling that later began to ooze, resolving into a chronic ulcer, sometimes under-

[2] Strong, R. P., *This Journal* (1906), 1, 91.

[3] Stitt, E. R., *Journ. Cut. Dis.* (1908), 26, 103; also ref. *Journ. Am. Med. Assoc.* (1907), 49, 1305.

mined, sometimes punched out. Deep scrapings showed no pyogenic organisms and practically no polymorphonuclear leucocytes. Lymphocytes predominated with many large cells, 20 to 30 microns in diameter, some of which looked as if they were full of small circular bodies, which, however, showed no chromatin staining after Giemsa's stain. Healing was very slow, and treatment was unsatisfactory. The second type was characterized by an acute exudate with a dirty membrane and irregular-staining diphtheroid bacilli.

Shattuck,[4] at the same meeting, reported a series of 35 cases of chronic Philippine ulcers, none of which was a typical "tropical ulcer" (phagedenic); all were of obscure causation. On clinical grounds solely, microscopic findings being negative or ambiguous, he concluded that there were four types. Syphilis was probably a factor in a few cases and possibly so in the great majority; others were not diagnosed. The diagnosis of probable syphilis was arrived at by exclusion, not by positive findings, and in spite of atypical features. In the group of 19 cases from Catbalogan, Samar, the resemblance between the different individual cases was very striking and pointed to a common etiology, although the lesions ranged from simple ulcers to contractures and loss of toes and bone destruction. The possibility that any of these may have represented late yaws was not discussed.

GENERAL DESCRIPTION

The present report includes six cases that were seen in the clinic of the Sanitary Survey Commission No. 2 of the Philippine Health Service, during its survey of Hagonoy, Rizal, by Dr. T. P. Corpus, the Commission surgeon, acting under instructions to note particularly all cases suggestive of blastomycosis or any allied condition. Some were seen by me once, others twice. Material for study was collected each time, and on the second occasion photographs of four of them were obtained. More extensive study of the cases themselves was prevented by prevailing circumstances.

GROSS LESIONS

The lesions varied greatly in duration, extent, and activity, yet presented features that suggest a common etiology. However, they possessed no peculiarity that could differentiate them distinctly as a clinical entity. They developed subcutaneously, sometimes as a pustule, usually as a firm nodule that broke

[4] Shattuck, R. R., *This Journal, Sec. B* (1907), 2, 551.

down after an indefinite period. Usually they were not in themselves painful, though sensitive to manipulation.

Shallowness of the ulcer itself and fairly extensive undermining of the skin along an advancing border (as in fig. 2, etc.) were features of the superficial lesion. Frequently, however, deeper structures had been more or less promptly invaded, with disturbance of function and, in one case (fig. 5), shortening of an affected member. Fusion of separate ulcers or progression of lesions irregularly outward with cicatrization of the older central area had given rise to considerable irregularity of outline of the affected areas. Induration of the advancing margin was usually noticeable, though skin that had become well undermined might on the surface still appear practically normal. The floor of the ulcer was usually covered with a thin layer of pale necrotic material. Beneath this was a shallow, pale granular layer of invaded subcutaneous tissue, more or less soft and friable, sometimes exuberant and vascular, bleeding freely on manipulation. Those lesions invading deeper structures could not be investigated.

The process had sometimes subsided greatly only to break out afresh, with activation of indolent lesions and development and comparatively rapid extension of new foci. In other cases it had progressed slowly for months and years. It had usually resulted in more or less destruction and cicatrization of the soft parts (fig. 6), though in one case there was considerable thickening of the foot, somewhat suggestive of a mycetoma.

I am informed that administration of potassium iodide in large doses caused improvement in the cases so treated; however, the period of observation after treatment was begun was too short for results to be apparent. Conditions have prevented subsequent observation.

MICROSCOPIC FINDINGS

Smears.—Nothing has been found in stained smears to which the causative rôle could be possibly assigned. In those from superficial levels bacteria of different types are found, though they are not numerous. Polymorphonuclear leucocytes usually predominate. In smears from the deeper levels bacteria are scarce when found, and cells of the lymphoid type usually predominate. Eosinophiles are sometimes numerous.

The only objects of interest observed with any regularity are small, round or oval bodies, staining deeply with Loeffler's blue or Giemsa's stain, apparently produced by fragmentation of leucocytes. In some preparations they are scarce, and in others

they are fairly numerous. Most of them measure 2 to 3 microns in diameter. They usually lie singly; sometimes they lie in pairs. Occasionally a medium-sized body is surrounded by a zone of faintly staining protoplasm.

The formation of bodies presumably identical may be sometimes seen in a polymorphonuclear leucocyte the nucleus of which is breaking up into small, round, clear-cut masses ("chromolysis"). There are usually 3 to 6 of these, measuring 2 to 2.5 or 3 microns in diameter, though when but two are formed they may be 3 to 4 microns. Once liberated by breaking down of the cytoplasm, they are no longer identifiable as of leucocytic origin.

Sections.—Too little tissue has been examined to establish a typical histology. The lesion is primarily a chronic inflammatory infiltration of the chorium, with lymphoid and, more prominently, plasma cell accumulation. Eosinophiles are usually present and sometimes numerous. Abnormal accumulation of the normal pigmented cells of the chorium is sometimes seen.

Proliferative changes are sometimes evidenced by numerous prominent blood vessels, of small lumen but thick walls of prominent cells rich in protoplasm; these lie in a cellular connective tissue. Endothelial proliferation may diminish the lumen (figs. 9 and 11), sometimes obliterating it entirely and producing an area of endothelial cells that may suggest an early tubercle (figs. 10 and 12). In two areas of one section there were found a few giant cells with nuclei circumferentially arranged (fig. 17). As a whole, this process undoubtedly tends to make the tissue liable to degenerative changes. Acute inflammation, with infiltration and subsequent necrosis, apparently develops secondarily, though the causative agent is not evident. It may be seen developing in and about small vessels, in which thrombosis may occur (fig. 13), dependent upon inflammatory injury of the vessel, as in figs. 14 and 15.

The epidermis is usually somewhat hypertrophic. In one instance (fig. 7) it is peculiarly canalized by the papillæ, which sometimes extend very close to the surface. Pigmentation is diminished where hypertrophy is evident. The epidermis is not invaded, even at the edge of the ulcer. Loss of its integrity seems to be dependent on nutritional disturbances caused by the underlying lesion (fig. 8).

Few organisms of any sort can be found in appropriately stained sections, even in the acutely inflammatory areas, and none can be found in the deeper zones. That the acute reaction always results from secondary bacterial invasion seems, from this and the number of bacterial colonies that appeared in the

cultures, an inadequate explanation. There are sometimes found areas containing numerous minute to coccoid or larger granules and prominent shreds that stain intensely with basic dyes, as if of nuclear material (figs. 13, 14, and 15). Their source is not evident, unless they result from chromolysis of leucocytes, yet they are found in areas too recently invaded for necrosis of the leucocytes ordinarily to be expected.

In most sections nuclear changes in the leucocytes are noticeable. Among typically stained lymphocytes there may be more or less numerous round, solid, intensely staining bodies of the same size, apparently pycnotic individuals. In the acute exudate, both in the recently invaded areas and in regions of extensive infiltration and histolysis (figs. 18 and 19), polymorphonuclear leucocytes undergo, instead of ordinary necrosis, globular fragmentation (figs. 20 to 28, inclusive), by which the nuclei separate into 3 to 6 or 8 discrete, round, dense bodies, which lie in well-outlined pale red protoplasm. The size generally varies inversely with the number; occasionally there are as many as 12 to 15 fragments that are mere granules. This is evidently the same process as seen in the smears. The masses apparently do not long survive as such after the protoplasm breaks down, for free bodies are nowhere numerous.

In some cells there are but two bodies (figs. 20 and 27), and in others but a single, round, comparatively large nuclear mass. In its size and in the appearance of its protoplasm this mononuclear cell is similar to the multinucleated cells; it appears to be a polymorphonuclear the nucleus of which has fused into a solid, round mass.

CLINICAL DIFFERENTIATION

In certain clinical features, but especially in the impossibility of establishing a definite clinical diagnosis, this ulcer is like certain of those reported by previous authors. Leprosy, tuberculosis, discomycosis ("streptothricosis"), and "blastomycosis" of any type may be ruled out on the absence of the specific organism, as is dermal leishmaniosis, or oriental sore, which is not known to exist in these Islands. The lesion is clinically unlike the syphilitic ulcer, and the patients denied having had primary or secondary lesions.

The two lesions that have particularly to be differentiated are the tropical ulcer and late yaws. The former is rather the more similar and is not positively differentiable on clinical grounds, though those cases of tropical ulcer that I have seen usually did not exhibit the undermining so prominent in the

lesions under consideration. Neither the spirochæte of tropical ulcer nor the fusiform bacillus usually associated with it could be found in smears from these ulcers.

Tertiary yaws is, at least in the oriental tropics, very apt to be invoked in explanation of such lesions as the more advanced of these cases, and it is very probable that in yaws districts similar lesions, because of their negative microscopic findings and the beneficial effect of treatments that include potassium iodide, might be so diagnosed. I am strongly of the opinion that late yaws can be eliminated in the present instance because of dissimilarity of its development, course, and general appearance; negative history and absence of evidence of early yaws in these cases; and freedom of the immediate district from the disease. Negative search for the treponema in the chronic cases is not valid evidence in this consideration.

It is, therefore, probable that these lesions were due to some unknown infective agent, not demonstrable in smears or sections, but characterized by persistence in strictly localized lesions over long periods, and capability of causing considerable damage to the individual. This being borne in mind, the curious features of the cultural findings can be accepted as of more significance than might at first seem possible.

CULTURAL OBSERVATIONS

Utilizing tissue fragments and scrapings from incisions, a large number of cultures were made. The media used included ordinary nutrient agar, with and without dextrose, maltose, and glycerin, and a number of special fruit media. On none of the ordinary media and in no culture planted with fluid material were developments of interest observed. In several cultures mold fungi developed, evidently from spores contaminating the lesions. Bacterial contamination was practically constant, though in cultures of material from deep in the lesions there were few colonies. In most cultures the tissue elements were rapidly destroyed, as was to be expected from the combination of autolysis and bacterial overgrowth. However, in tissue planted on certain of the special media there occurred, whether in spite of, or dependent upon, the influence of the accompanying bacteria, certain changes in the leucocytes that are distinctly unusual and of considerable interest.

HYALINIZATION IN NUCLEI OF LEUCOCYTES

The term hyalinization is used as descriptive of the appearance in fresh preparations of the leucocytes whose nuclei, wholly

or in part, became firmer, larger, and more glistening and that separated, sometimes as if by fracture (fig. 31), into two or three distinct masses or fragments. In stained smears these masses usually showed well-defined outlines, with wide variation in color. The more advanced were oval or rounded and deeply stained. They were more or less differentiated from those nuclei, or parts of nuclei, that were not so intensified (figs. 29 to 38) and that sooner or later degenerated. Certain cultures in which bacteria did not multiply seemed unfavorable for this nuclear change, though it was apparently attempted (figs. 41, 42, and 43).

BASIC FORMS

These bodies are round, oval, or lenticular, sharply outlined, solid, intensely stained by Loeffler's blue and deeply stained by Giemsa's, without any determinable structural differentiation, and range in size from 2 to 4 or 4.5 or from 3.5 or 4 to 5 or 6 microns in different cultures (figs. 39 and 45). Because of their essential resemblance to the bodies to which I applied the term "basic form,"[5] they will be spoken of by that name. They were found only in cultures of tissue fragments on the fruit media and were never numerous. Bacterial growth seemed to facilitate their development, if not to be essential.

Their appearance in fresh preparations and stained smears indicate that they develop from the more deeply staining of the nuclear elements just described. They are often by no means sharply differentiated, for in a single preparation the gradation from pale degenerate cell fragments to intensely stained basic forms is not abrupt, though the range is usually wide.

The ultimate end of the basic forms is not evident from their appearance. Though in certain instances there was a strong suggestion of multiplication by fission, this cannot be asserted to have occurred. It is certain that their production was neither active nor sustained, and they more or less slowly disappeared.

CRYPTOCOCCUS

In one culture from each of cases I and II, there were found, after seven months and one and a half months, respectively, definitely differentiated fungous organisms, unicellular, of somewhat yeastlike morphology. These elements were small, usually measuring between 1.3 by 1.6 and 2.0 by 2.5, rarely 3.0, microns. They were sharply outlined, never doubly contoured, but often surrounded by an unstained halo, probably due to shrinkage

[5] *This Journal, Sec. B* (1916), 11, 267.

rather than to a special capsular structure. Wide variation in density of staining was often apparent. Young individuals (and particularly those in case I) tended to stain solidly (figs. 51 and 52). Older organisms (and particularly those in case II) were often granular (figs. 54, 55, and 56), some granules staining red by Giemsa's. In many groups some were unstained (fig. 59), appearing in stained, wet-mounted preparations as colorless hyaline bodies.

Multiplication took place by a peculiarly clumsy gemmation that sometimes approached binary fission (figs. 54, 55, and 57). It was evidently a very slow process, for though many individuals exhibited it, the numbers of cells produced were not great. Microscopically masses of them were found (fig. 49), but nothing approaching visible colonies was ever attained.

Cultivability was so low that in no transplant, even on the media ordinarily most favorable to fungus growth, was any development observed. It seemed as if the remnants of tissue elements were essential to their multiplication. When this was dispersed on fresh media, or exhausted in the original cultures, growth ceased, and the organisms did not long survive. It is possible that, together with special suitability of the medium (nutrient prune agar), symbiosis with the accompanying bacteria also may have had an influencing rôle in their development.

DETAILS OF CASES AND CULTIVATION WORK

In these reports the common descriptions, including the findings in smears and in sections of tissue fragments where such were examined, will not be detailed.

CASE I

"Mycetomalike" foot with ulcers.—M., Filipino rice farmer, aged 56 years, from a barrio somewhat distant from Hagonoy, seen August 17, 1916. The condition began with a painless soft tumor on the dorsum of the left foot two years ago. This did not ulcerate at first, but extended in the deeper tissues, the skin later breaking down and producing an ulcer that slowly enlarged, finally healing for the most part. When seen, the foot and ankle were thick and heavy, with marked induration extending from just behind the toes to above the ankle. The whole was rather suggestive of a mycetoma, except that there were no sinuses or discernible subcutaneous foci of necrosis. No bone destruction was evident. An area of skin over the dorsum was ulcerated, with scar tissue about it. Above this, on the ankle, was a large fungating ulcer (6 to 8 centimeters in diameter) said to be of about one year's standing. The floor was made up of soft pale granulations, from which material was removed for cultures and sections. I was unable to see this patient again, but the Commission surgeon reported that under potassium iodide the active lesions subsided completely.

Small pieces of tissue from three points in the lesion, superficial and deep, were planted (August 17) on nutrient banana agar and on banana cylinders. In smears (October 4) from the material on the latter, bodies of the cell-fragment and basic types were particularly noted, though they were at that time considered insignificant elements of tissue degeneration.

No further development having occurred, on November 18 (three months after planting) the deep-tissue fragment on banana cylinder was removed, crushed in a drop or two of sterile bouillon, and distributed to eight slants of special media. These included nutrient prune and nutrient banana agars (some with maltose added) and ordinary 2 per cent maltose and dextrose agars. The last two cultures quickly became overgrown with bacteria, among which very few cell fragments could be found; they were finally discarded as negative (January 3, 1917). In the fruit agar cultures (November 24) numbers of the forms were found, basic forms among them, particularly in the two on nutrient prune. On January 3 they had further increased in these, but were less numerous in the others. The material from several tubes was then transferred to tubes of plain (nonnutrient) banana agar slants, the parent tubes being kept for further observation.

In the subculture from maltose nutrient prune the various forms were numerous on January 17, and at several points in the smear there was a distinct grouping of the basic forms, as if they had multiplied in a mass. The other subplants showed few forms of interest. All were now sealed with paraffin and put away at body temperature.

On March 11 the smears from parent tubes (from which the November 24 transplants had been made) showed occasional small, round, basic forms on maltose nutrient prune, a very few apparently dividing by an indistinct process of fission. There were a few rounded and oval basic forms and numbers of the less definite cell-fragment bodies on nutrient banana.

In one nutrient prune agar culture there was found, among the staphylococci and fairly numerous cell-fragment and basic forms, a *Cryptococcus* essentially similar to that previously found in a culture in case II. In the smear it occurred singly and in small groups (figs. 51 and 52), though sometimes in larger masses (fig. 49). Nothing approaching colony formation could be detected.

The January 3 subplants now showed less numerous forms of interest than when last examined. Subplants were made from the two most promising of them, and a guinea pig (G. P. 73)

was inoculated intradermally. Transplants were also made from the *Cryptococcus* culture to several serum-containing agars. No evidence of further development was observed. On May 28 there remained insufficient *Cryptococcus*-containing material for further study, and all tubes were discarded.

CASE II

Ulcers and scars of hands and wrist.—K. M., Filipina, age 20 years, a laundress, seen August 17, 1916. On the back of the left hand was an old depressed scar from an ulcer that had begun two years before and persisted for several months, and in the palm of the right hand (fig. 2) was the scar of an ulcer that healed about a year previously. The active lesions were on the right hand and wrist; dorsally there was an irregular, apparently conglomerate ulcer extending to the fingers, of about one year's duration, apparently involving the deeper structures, the second and third fingers being maintained, though not rigidly, in flexion. Anteriorly on the wrist was another active ulcer, apparently confined to the superficial tissues.

On November 17 the dorsal ulcer (fig. 3) showed little change. The wrist lesion (fig. 2), however, was extending rapidly; above it was a new lesion, but a month old, that was typical, with undermining of the skin at the advancing border, an irregular, granular, very vascular base, and scar formation where the activity had subsided.

A fragment of tissue excised on August 3 from the wrist ulcer (lower) was planted on a banana-pulp cylinder. It proved to be badly contaminated. On November 17 tissue fragments and scrapings from superficial and deep levels of the newly developed, active ulcer were planted on various media (culture series 1 and 2).

Culture series 1.—This series included six cultures on nutrient banana and nutrient prune agars planted with small tissue fragments. On November 25 two were heavily overgrown with molds and bacteria and two others contained little but bacteria; these were discarded. A smear from a deep-tissue fragment on nutrient banana agar contained numerous small masses and bodies ranging from very pale indefinite fragments to clear-cut, densely stained bodies. Many similar forms were found on nutrient prune agar. The material from the nutrient banana culture was removed and distributed to culture series 3 and to fresh nutrient banana and prune slants; the latter were made anaërobic.

Culture series 2.—These cultures, planted simultaneously with series 1 with triturated tissue from deep in the lesion, included, among others, one slant each of: (1) Plain and (2) maltose nonnutrient prune, (3) plain and (4) maltose nutrient prune, (5) plain and (6) maltose nonnutrient banana, and (7) plain and (8) maltose nutrient banana agars.

In most of the cultures bacterial colonies appeared, though they developed poorly on the nonnutrient prune and banana agars. In smears from several cultures there were found occasional forms more or less approaching the basic type among the bacteria and irregular cell fragments, which were at first rather numerous. On December 17 they were still numerous on several media, though generally diminished. On January 3, 1917, the above described *Cryptococcus* was found in the culture on nutrient prune agar (medium 3). This was the first time that it had been observed. On medium 4 there were few of the cell-fragment forms, on 5 and 6 numbers of them were found, and on 1, 2, and 7 they were fairly numerous in the heavy bacterial growth.

The *Cryptococcus* was more plentiful on February 6, though nowhere was there a suggestion of colony formation. Various cell-fragment forms were still present in most of the other cultures. In culture 6 they seemed to have increased in numbers. In culture 4 some appeared to be attempting to differentiate into the *Cryptococcus;* this, however, did not materialize.

On March 11 the various formed elements having distinctly diminished, transplants were made to other special media, and material containing small numbers of the *Cryptococcus* was inoculated into a guinea pig (G. P. 44) intradermally and a rabbit (R. 43) intraperitoneally in a porous capsule. The results were negative.

Culture series 3.—On November 25 the material from a nutrient banana culture (culture series 1) was distributed on the same media as in series 2. The results are of interest only in that, starting with a uniform suspension, the forms here practically disappeared except on media 1 and 5, where they seemed to multiply temporarily. The anaërobic cultures were negative.

CASE III

Multiple ulcers of right leg and foot.—L. A., Filipina, age 14, seen August 17, 1916. Occupation, housework; seldom in fields. The primary lesion had appeared about a year previously on the right small toe (fig. 5), which had become thickened, decidedly shortened, and was superficially ulcerated. The evident involvement of the deeper tissues, with but a very shallow ulcer, was noteworthy. The consistence of the exposed tissue could not be investigated, the lesion being very sensitive.

On the inner surface of the leg were two groups of ulcers more or less separated by normal skin and scar tissue. The first had appeared seven months previously. Anteriorly on the ankle was an abruptly raised lesion 3 to 4 centimeters in diameter. The skin at the edge was not indurated, but was turned abruptly outward, as if by a draw string, for about 0.8 centimeter. The plateau thus produced consisted of granular friable in-

flammatory tissue. On the inner surface of the foot was a firm swelling 3 by 5 centimeters, evidently a developing focus, covered by intact skin; this was of several months' duration. Material for cultures and smears was removed from open ulcers, but incision of the unopened mass was not permitted.

On November 18 the lesion over the ankle had lost its fungating character (figs. 4 and 5), the mass on the inside of the foot was represented by irregular ulcers, and those on the leg (fig. 4) had enlarged and were more fused. The condition, as a whole, seemed more active. Small fragments of superficial tissue were again secured.

On August 17 material from three different lesions was planted on nutrient banana agar and banana cylinders. The cultures became overgrown with bacteria—*B. pyocyaneous* and *Staphylococcus aureus* chiefly—and molds and were discarded.

On November 17 other tissue fragments were obtained from two active ulcers, though not sufficiently deep for satisfactory cultivation work, and plants were made on various media. On November 25 numbers of forms approaching the basic were found in several cultures, few in others. On January 2, 1917, there was apparently no increase of these bodies where they were to be found. From three of the possibly favorable cultures the material was transferred to plain banana agar slants. On February 10 a very few basic forms were found in one of the subcultures. Subplants from these to special media, and a guinea pig (G. P. 110) inoculated intradermally, gave no results.

Comment.—Though the lesions in this case were fairly active, satisfactory material was not obtained. The lesions seemed very sensitive, and the patient was unusually timorous. Being ignorant and careless, she had neglected the lesions, and contaminating organisms were particularly numerous.

CASE IV

Chronic ulcers of left leg, with deep scars.—The patient, T. P., a Filipino, farmer, aged 19 years, was seen November 17, 1916. He was somewhat undersized and not robust. The condition had begun eight years before as a small sore at the knee. This had spread, evidently superficially, downward over the entire inner surface and much of the outer, leaving a soft, thin, parchmentlike skin extending to the inner malleolus.

On the inner surface of the lower half of the leg were three irregular ulcers (two shown in fig. 6) in an area of marked tissue loss. This is poorly seen from the angle at which the photograph was taken. Fibrosis was extensive and involved the muscular tissue, with some limitation of motion.

The ulcers were fairly typical, extending outward in the more or less fibrotic tissue, with slow undermining of the epidermis (fig. 8) and induration of the overlying layer. Healing occurred centrally as the lesions progressed outwardly, leaving scar tissue (fig. 6). These lesions were said to have been active for two years, but it could not be determined positively

whether this was a new infection on the superficial scar of the original lesion or whether it was a renewal and extension of the latter. The progress of the infection was very slow; the Commission surgeon reported that the lesion was practically unchanged in three months.

Tissue for cultures and sections was removed from the edges of the most active-looking lesion, and smears were made from several points. These show no special feature on microscopic examination.

Two tissue bits from deep in the lesion were each divided and planted on nutrient banana and nutrient prune agars. Bacterial growths promptly appeared in all. On November 24 smears of the softened tissue on nutrient banana showed, among the staphylococci, many cell fragments and densely staining forms. Very few of the latter were found. In transplants from some of these cultures nothing but bacterial growth developed, and they were finally discarded.

After six weeks on one of the original nutrient prune cultures the fragment of tissue was removed (January 2) and triturated. Occasional clear-cut basic forms and numerous more or less fragmented cells, numbers of them deeply staining, were found. Subplants of this material were made on plain and nutrient banana agars. A rabbit (R. 64) was inoculated in the anterior chamber; it died in three days, apparently from staphylococcic invasion. On January 17 the plain banana subplant showed numbers of small, usually round, basic forms. The nutrient banana subplant was discarded because of contamination. No change occurring, further subplants to several other special media were made (March 13), another rabbit (R. 108) was inoculated, and two or three drops of sterile serum were added to the original culture. No further development occurred, and on June 28 all tubes were discarded. This rabbit remained negative.

Comment.—This case presents the lesions of longest standing in the series, the greatest area of involvement, and the most serious tissue loss. The lesions were very indolent and, therefore, presumably not rich in the infecting agent.

CASE V

Early lesion, right foot.—B. S., a Filipino, 25 years of age, farmer, in excellent general condition, was seen on November 17, 1916. Six weeks previously a small pustule had appeared at the center of the lesion presented (fig. 1). This had been scratched and frequently irritated and had slowly enlarged.

When seen, the lesion appeared as an area of superficial erosion without ulceration, measuring 4 by 5 centimeters, on and behind the inner malleolus. This area was rough, pale superficially, red in the deeper portions. At about the center (arrow, fig. 1) there was a slight opening that led into a flat, collapsed cavity. A probe inserted obliquely passed under the skin

for about 1 centimeter downward, forward, or upward. The process had confined itself to the subcutaneous tissue. Upon incising the overlying skin, the cavity was found to be lined by a thin layer of pale necrotic material over vascular inflammatory tissue.

Because of its situation no tissue was removed from the floor, and only a small piece was removed from the skin itself. Smears showed bacteria, but nothing else of interest. The tissue section is shown in fig. 7.

Small bits of tissue were planted on nutrient banana and nutrient prune agars. On November 25 both tubes showed bacterial growth, more on the banana than the prune. In smears from the former none of the cell-fragment forms were found, though a few were present on January 2, at which time the material was transplanted to plain and fresh nutrient banana agars. On January 17, these cultures being negative, they were sealed with paraffin and put aside at body temperature. On February 20 they were still negative and were discarded.

The nutrient prune culture had shown (November 25) a few suggestive forms. Transplants were made (January 2) from this to plain and (fresh) nutrient banana agars. On January 17 the nutrient banana subcultures showed heavy staphylococcus growth and practically no forms of interest. On the plain banana, however, on which the bacteria were largely inhibited, numbers of cell fragments were found. All tubes were paraffined and put away at body temperature. On February 20 only the two plain banana cultures showed a few cell-fragment forms; a few were still to be found on March 16, but attempts to stimulate development were unsuccessful. A rabbit (R. 111) was inoculated in the anterior chamber without result.

Comment.—This, the earliest case in the series, I found accidentally in the clinic as it was being dressed as an insignificant lesion by the clinic attendant. So far as the preliminary findings indicate, it is probably of the same nature as the above cases.

CASE VI

Inflammatory tumor beneath scalp.—The patient, Filipina, aged about 30 years, complained, when seen August 3, 1916, of a painful tumor of the size of a small olive beneath the scalp on the left side. This had troubled her increasingly for three weeks, finally preventing sleep. The mass was adherent to the bone, not to the scalp, and was painful, firm, not fluctuant. The patient presented no other lesions.

Exploration by needle elicited no evidence of necrosis or suppuration. Incision was not permitted. By aspiration two or three drops of bloody fluid were obtained. In a single culture on dextrose agar this was negative. Microscopically no organism was found. High lymphocyte and eosinophile content (above 10 per cent) suggested a chronic inflammatory condition.

Subsequently the mass was incised by the Commission surgeon, who found only well-vascularized tissue, apparently inflammatory. The condition not being relieved, he then infiltrated the mass with 95 per cent alcohol, 5 cubic centimeters, twice, two days apart. This caused acute pain, which slowly subsided, ceasing entirely after a few days, when the mass began to diminish. When the patient was next seen by me (November 17), there was no trace of the mass itself, but a distinct irregular, shallow area of depression, about 1 by 2 centimeters, could be felt in the bone. This evidently resulted from erosion by the tumor, and probably explains the severe pain.

Comment.—The etiology of this lesion is, of course, unknown. The efficacy of the treatment applied bespeaks an inflammatory process. The findings in the aspirated material (few polymorphonuclears and no organisms) and a negative culture are against a bacterial cause. The history, in ulcer cases, of similar nodules preceding ulceration, the apparent lymphocytic and eosinophilic infiltration, and the curious tendency to affect the bone, corresponding to deep invasion in some of the ulcer cases, makes this lesion of interest as possibly of the same nature.

SUMMARY AND DISCUSSION

In the cases detailed above, search along usual lines for a demonstrable causal organism of recognized type, whether bacterial, protozoal, or fungous, has been totally without result. The lesions, which present clinical evidence of etiological similarity, are clearly not bacterial, leishmanial, or of the ordinary tropical phagedenic type. There is no positive evidence that it is of syphilitic or frambœsial origin, nor is a treponema demonstrable in scrapings. Clinically, therefore, it seems to fall into the category of ulcerative and destructive processes of unknown etiology.

Intensive cultural study of excised tissues has resulted in observations that are suggestive of a hitherto unrecognized process of infection. On ordinary media bacterial overgrowth was always prompt and the tissue elements rapidly degenerated; no special feature was seen. On special media, however (nutrient banana and, particularly, nutrient prune agars), leucocytes of the planted tissue fragments instead of disintegrating underwent, in spite of the combined influence of autolysis and bacterial overgrowth, a change unlike that ordinarily to be seen in such material. This was a peculiar solidification (suggesting hyalinization) that the nuclei underwent, whereby they or parts of them became more deeply staining. This is no doubt in part due to the peculiar effect of these media, on which tissue autolysis is often considerably inhibited. However, apparently by further

changes in the denser of these nuclear fragments there developed bodies that are, I believe, not referable to changes induced by the media alone. These were firm, oval or round bodies, deeply staining by Loeffler's methylene blue and similar stains, clear-cut in outline, an appearance that suggested parasitic fungous elements.

In one culture only from each of two cases of the series there was finally found a definitely fungous element, a *Cryptococcus*.[6] In one case it was found after cultivation for six weeks on nutrient prune agar, but in the other only after seven and a half months on culture media, three months on a banana cylinder, and four and a half months on nutrient prune agar.

The source of the *Cryptococcus* is not clear. No similar organism is to be found in earlier smears from those cultures in which it developed. Difficult as it may be to accept, there seems to be a direct parental relationship on the part of the described basic bodies. Certainly it is not possible to differentiate between some of the typical basic forms and some of the more intensely stained fungus cells.

The significance of the fungus depends, of course, upon its source. On the evidence it cannot be proved to be essential in the lesion, though the conditions under which it developed led me to believe this to be the case. To establish this as a fact, cultivation of the organism in sufficient amount to permit the production of experimental lesions with it would be necessary. Though the material available, in large part bacterial growth, was expended entirely in attempts at subcultivation, this was not accomplished. Animal experimentation could, therefore, not be attempted.

To deny this organism an essential rôle, one must assume it to be a contaminant, either of the lesions or of the two cultures involved. If it was in the original material as removed from the lesion, it certainly was not evident at the time. Furthermore, had this been the case, it or some recognizable precursor should have appeared earlier in those cultures containing it and in more of the cultures made from the tissue fragments in those cases.

[6] The genus *Cryptococcus* Kütz. 1843 includes organisms that reproduce by budding only; they do not produce mycelia, or spores. In this group fall, in default of essential information, the degraded yeastlike forms that may be adopted, with more or less permanency, by higher fungi of widely diverse classification.

The possibility that it is a laboratory contaminant of the cultures cannot, of course, be denied. However, in view of its characteristics, and in the light of experience with the conditions in this laboratory, this seems to me improbable. The cultures were paraffin-sealed, and at no time showed any evidence whatever of ordinary contamination. They at first contained only the curious cell-fragment forms among the bacteria, a staphylococcus in one case and a proteuslike bacillus in the other. After different periods of time the *Cryptococcus* was also found.

The fungus itself was unlike any of the many strains of yeastlike organisms of which I have found descriptions or have myself met with. I have never seen any laboratory contaminant that resembled it in the slightest, and it is totally unlike the fungi of that class that I have from time to time cultivated from sputum, urine, fæces, fruit, etc., here and elsewhere. Its characteristic features of nonproduction of visible colonies, of an unusual and evidently very slow process of multiplication, of noncultivability in fresh subplants away from the remnant of the tissue material originally planted, though put on media particularly adapted to the cultivation of fungi, are not suggestive of a saprophyte but, on the contrary, bespeak a highly parasitic organism.

The alternative that the observed fungus is essential to the lesion seems, therefore, quite possible. The appearance of the lesion itself and its course and reaction to potassium iodide are indicative of mycotic infection. Though no fungus element can be demonstrated in preparations from it, the same is true of other microörganisms. The fungus that was found appeared only in cultures of material from two clinically active cases, on a medium (nutrient prune agar) that is especially favorable to the growth of fungi. The characteristics of the two strains seem to be identical and are essentially those of a highly parasitic organism, as might be expected of the causal agent of such lesions. Therefore the weight of evidence, positive and negative, justifies the serious contemplation, if not the assumption, of the conclusion that the described fungus element represented the infecting organism.

From the mycologist's point of view the *Cryptococcus* is, undoubtedly, to be regarded as a degraded form of a higher fungus. From the viewpoint of the hypothesis outlined above, however, assuming that it is the causal organism, it is apparent that it may

actually be a comparatively highly differentiated phase of an organism not to be demonstrated morphologically in the lesion to which it gives rise.

In what form such an organism would maintain itself in the tissues is not evident from the findings. If it is as a formed body, it probably resembles so closely some tissue cell as to be indistinguishable morphologically or tinctorially. The alternative is that it occurs as a body or substance with little or no morphological differentiation, whether maintained intra- or extra-cellularly. The observed development of the peculiar basic forms from tissue elements in cultures, in conjunction with certain of the findings in the lesion, suggests the existence of an amorphous intracellular substance that is passed on from cell to cell by disintegration of those infected. The granules and shreds seen in some of the tissue sections (as in figs. 13, 14, and 15), and the peculiarly orderly fragmentation of degenerating leucocyte nuclei sometimes seen in older areas (figs. 18 to 28), might play a part in such a process. Such a substance would be, in effect, a localized, nondiffusible "virus" and would perhaps be midway between organisms of fixed morphology and the recognized filterable viruses that are known only by their constitutional effects.

The difficulty of determining whether or not the tissue or cultural cell fragments are of pathogenic significance, and not simply negligible degeneration products, is obvious. The fragmentation seen in sections, though interesting, is by accepted standards ascribable solely to pycnotic degeneration (chromolysis) and does not suggest any vital process. Further, in preparations from cultures none but the clear-cut basic forms are at all convincing as of possible significance; the lesser forms usually appear as negligible fragments of tissue cells that are often seen on the special media employed. However, the development of the denser forms and their persistence in spite of very unfavorable influences where developed distinguish them as of more than incidental importance.

The contagiousness of this infection is very slight or nil. No history of contact with previous cases or of subsequent infection of other individuals could be obtained. Were the exudate very infectious, this would be a simple matter because of intimacy and extreme hygienic simplicity of barrio life among this class of Filipinos. From the viewpoint of the suggested etiology of the lesion it may be that the invader, after adaptation between itself and the host became established, and while losing its mor-

phologic identity, also lost its ability to infect a fresh individual, or to return to a saprophytic, differentiated form. This seems entirely possible in view of the profound, sometimes essentially (ultimately) fatal, modifications that may be undergone by such organisms in adapting themselves to animal parasitism.

The hypothesis on which the suggested interpretation is based may seem fantastic. Nevertheless the observations described and those yet to be reported seem to permit of but one other possible explanation. This involves the recognition as pathogens of organisms belonging to the peculiar borderline group that includes the Myxomycetes or Mycetozoa, which are not recognized as of any importance whatever in animal pathology. For the present it will suffice to call attention to the possibility. The hypothesis adopted, based on modification of true fungi, seems at this time to be the more conservative and the more probable view. However, the distinction is not fundamental, for some of the lower fungi (such as the Chytridineæ) and the Myxomycetes are in some respects closely allied.

Of whatever type the invading organism may finally prove to be, the process of "cryptoplasmic" infection outlined is based primarily on observations and should be correct. At all events, there is presented a most baffling problem, one that is not easy to approach. Because of its essential peculiarities it will be very difficult definitely to establish it. On the other hand, since negative results are often inconclusive, it will be equally difficult positively to disprove it.

CONCLUSIONS

There has been found in one district of the Philippine Islands a type of lesion, invasive, and usually more prominently ulcerative, of undetermined etiology. On clinical grounds it seems very possibly mycotic. No parasite of any type, protozoal, bacterial, or fungous, can be demonstrated in the lesion. Fragmentation of the nuclei of polymorphonuclei into separate rounded masses ("chromolysis") is sometimes interestingly prominent.

In certain cultures on special media, of tissues excised from the lesion, the nuclear bodies of leucocytes have undergone modification to produce clear-cut, intensely staining "basic" forms. From each of two cases, in one culture only, there has developed a *Cryptococcus* of very low cultivability and of cultural requirements suggesting high parasiticity. This is thought not to be a contaminator but, in the absence of any recognizable precursor, possibly to be a development of the basic forms.

This suggested evolution can be explained only on the basis of the hypothesis that the infecting organism may have assumed a cryptoplasmic state on adapting itself to animal parasitism, followed by the invasion and utilization of the tissue cells, particularly the polymorphonuclears.

The general hypothesis as outlined has been formulated as a possible explanation of these and other more or less related observations, which it is the intention to report in the series of articles undertaken.

ILLUSTRATIONS

PLATE I

FIG. 1. Early lesion, duration six weeks. Before ulceration. Considerable undermining of skin with but a small sinus. Case V.

2. Ulcers of wrist; upper lesion unusually active, duration one month; lower lesion several months. Scar in palm from previous lesion. Case II.

3. Spreading lesion on dorsum of same hand, same case. Duration about one year.

4. Lesions of leg, case III. Irregular ulcer on inner surface of foot marks the site of a smooth swelling, skin intact, seen three months previously.

5. Original lesion, case III. Involvement of bone with some shortening of toe. Duration about fifteen months.

6. Lesions of leg, case IV. Full extent of tissue loss not apparent at the angle shown. Duration at least two years.

PLATE II

FIG. 7. Section from advancing edge of leg ulcer, case IV (see fig. 6), showing hyperplasia and canalization of epidermis, with absence of infiltration. × 11.

8. Similar section from case I. Thinning of epidermis at edge of ulcer evidently secondary to inflammatory lesion below. × 12.5.

9. Endothelial proliferation in small blood vessel, without infiltration. × 300.

10. Tuberclelike focus apparently marking site of small vessel. × 300. (Figs. 9 and 10 from same section as fig. 7.)

11. Vessel in center shows both proliferation and early leucocytic infiltration. × 180.

12. Focus of endothelial cells, with a few leucocytes, apparently resulting from obliteration of a vessel. × 180.

13. Vessel on left contains red blood cells; lumen of that on right almost obliterated; that in center obliterated by a thrombus containing small, deeply stained granules and fragments. × 180.

14. Area of small vessels with considerable leucocytic infiltration. Deeply stained granules (nuclear fragments?) and shreds are prominent. × 180.

PLATE III

FIG. 15. Area somewhat similar to fig. 14. × 300.

16. Large (mitotic endothelial?) cell seen in lower part of fig. 15. × 650.

17. Group of six Langerhans's giant cells. × 300.

(Figs. 11 to 17, inclusive, from deep tissue, case III.)

FIGS. 18 and 19. Exudate in superficial necrotic tissue from floor of ulcer. Normal-staining polymorphonuclear leucocytes contrasted to deeply staining and fragmenting cells. × 500.

FIG. 20. Contrasting cell containing two nuclear masses with ordinary, less densely stained polymorphonuclear. × 950.

FIGS. 21 and 22. Cells with three nuclear masses. × 950.

FIG. 23. Cell with six nuclear masses (one not visible in this plane). × 950.

FIGS. 24 and 25. Groups of small nuclear masses as deposited by breaking up of the cell. × 950.

FIG. 26. Large cell with six nuclear masses (one not seen), all distinctly crescentic (apparently degenerating). × 950.

27. Two nuclear masses in a leucocyte, density of staining much greater than in surrounding cells. × 950.

28. Two separate bodies, without protoplasm. Whether produced as in fig. 27 is not evident. × 950.

(Figs. 18 to 28, inclusive, from superficial tissue, case III.)

PLATE IV

FIGS. 29 and 30. Leucocytes separating into deeply staining lobules. Smear from culture, case IV, eight days on nutrient prune agar. × 1,000.

FIG. 31. Leucocyte dividing into three sharply edged fragments, of hyaline appearance. × 1,000.

32. Leucocyte producing two rather deeply staining bodies. × 1,000.

33. Leucocyte divided into three parts, one becoming oval and deeply staining, others soft, diffuse, pale-staining. × 1,000.

34. Oval body in amorphous remnant of cell. × 1,000.

FIGS. 35 and 36. Soft, amorphous degenerating cells. Clear-cut, round bodies (approaching the basic form) developed elsewhere. × 1,000.

FIG. 37. Large body apparently degenerating. × 1,000.

38. Free-lying body approaching basic. × 1,000.

(Figs. 31 to 38, inclusive, from smear from culture, case IV, eight days on nutrient banana agar.)

39. Typical sharply outlined, densely stained basic form. From case I, heavily contaminated culture on partially favorable medium. × 840.

40. Irregular mass of degenerating cell material, quite unlike the basic forms. From case I, heavily contaminated culture on unfavorable medium. × 840.

FIGS. 41, 42, and 43. Leucocytes with deeply staining nuclear masses in smear from tissue fragment, case I, triturated after three months on banana cylinder. Bacterial growth slight. × 840. This material transplanted to nutrient prune agar gave large basic forms; on other media it degenerated more or less promptly.

FIG. 44. Two forms approaching basic, apparently derived from one cell. × 840.

45. Clear-cut basic form. × 840.

46. Irregular body (approaching basic type) and very small clear-cut body suggestive of an organism. × 840.

PLATE V

FIGS. 47 and 48. Groups of more or less clear-cut, deeply stained cell fragments and basic forms. × 840.
(Figs. 44 to 48, inclusive, from case II, culture of tissue fragments eight days on nutrient prune agar.)

FIG. 49. Masses of cryptococci, case I. Culture of tissue fragment three months on banana cylinder and four months on nutrient prune agar. × 1,000.

50. Scattered cryptococci, case II. Culture of tissue fragments six weeks on nutrient prune agar.

FIGS. 51 and 52. Small groups of the cryptococcus, case I, same smear as fig. 49. Showing density of staining and irregularity in size and outline. × 1,150.

FIG. 53. Cryptococci, case II, from same culture as fig. 50. Showing (arrow) apparent attempt at mycelial growth. (This offshoot in the smear is clearly distinct from the surrounding bacteria.) × 1,300.

FIGS. 54, 55, and 56. Showing irregularity of size, shape, and mode of multiplication, neither clear-cut budding nor simple binary fission. × 1,150.

FIG. 57. Group of four sharply pointed apple-pip-like cryptococci. Their shape and close aggregation suggest origin by fission in two planes. × 1,150.

FIGS. 58 and 59. Cryptococci apparently degenerating, becoming pale staining in the former and unstained in the latter. Spaces believed not to represent organisms dropped out of smears. × 1,000.
(Figs. 54 to 59, inclusive, from same smear as fig. 53.) (Cultural forms all stained with Loeffler's methylene blue.)

PLATE I.

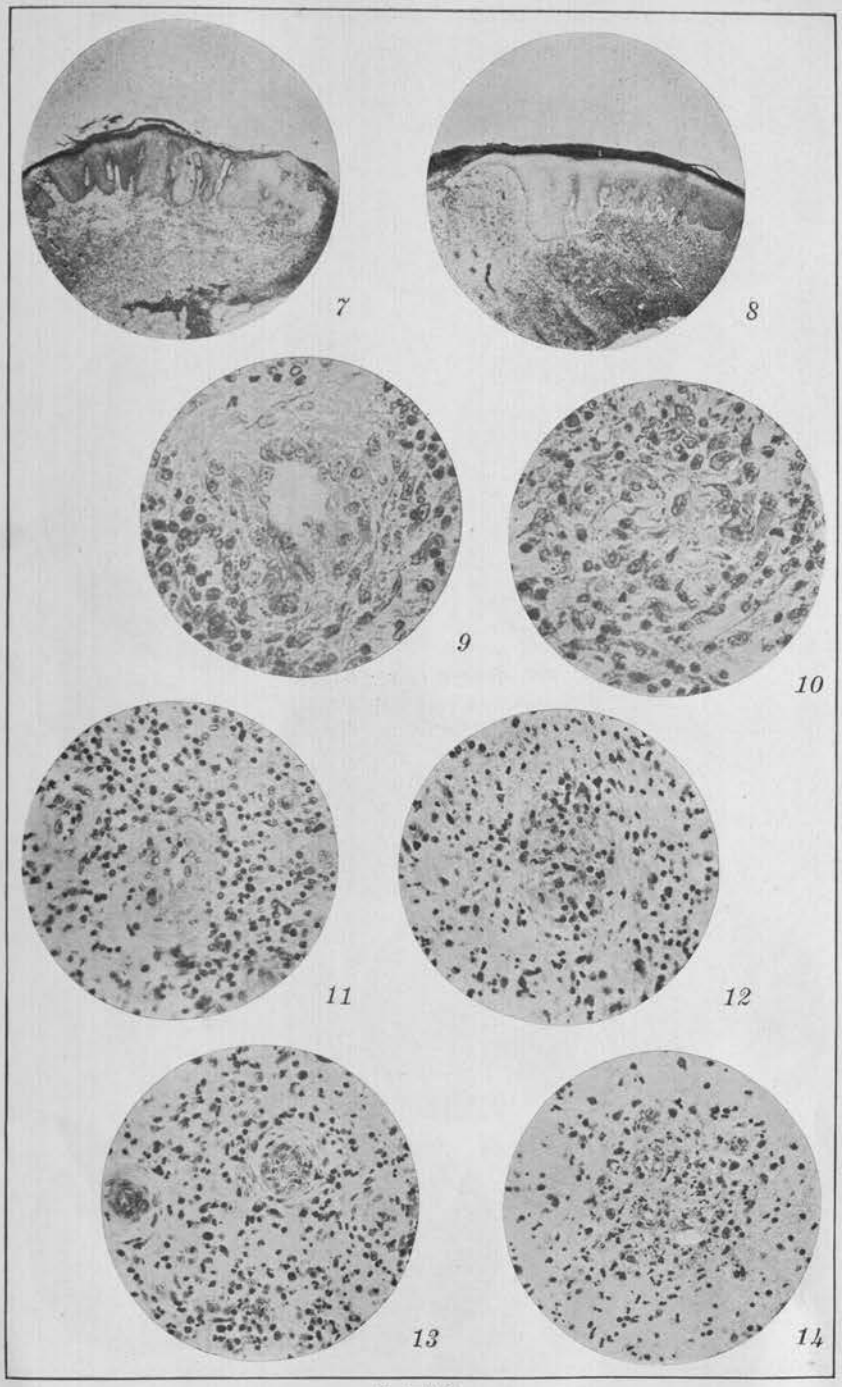

PLATE II.

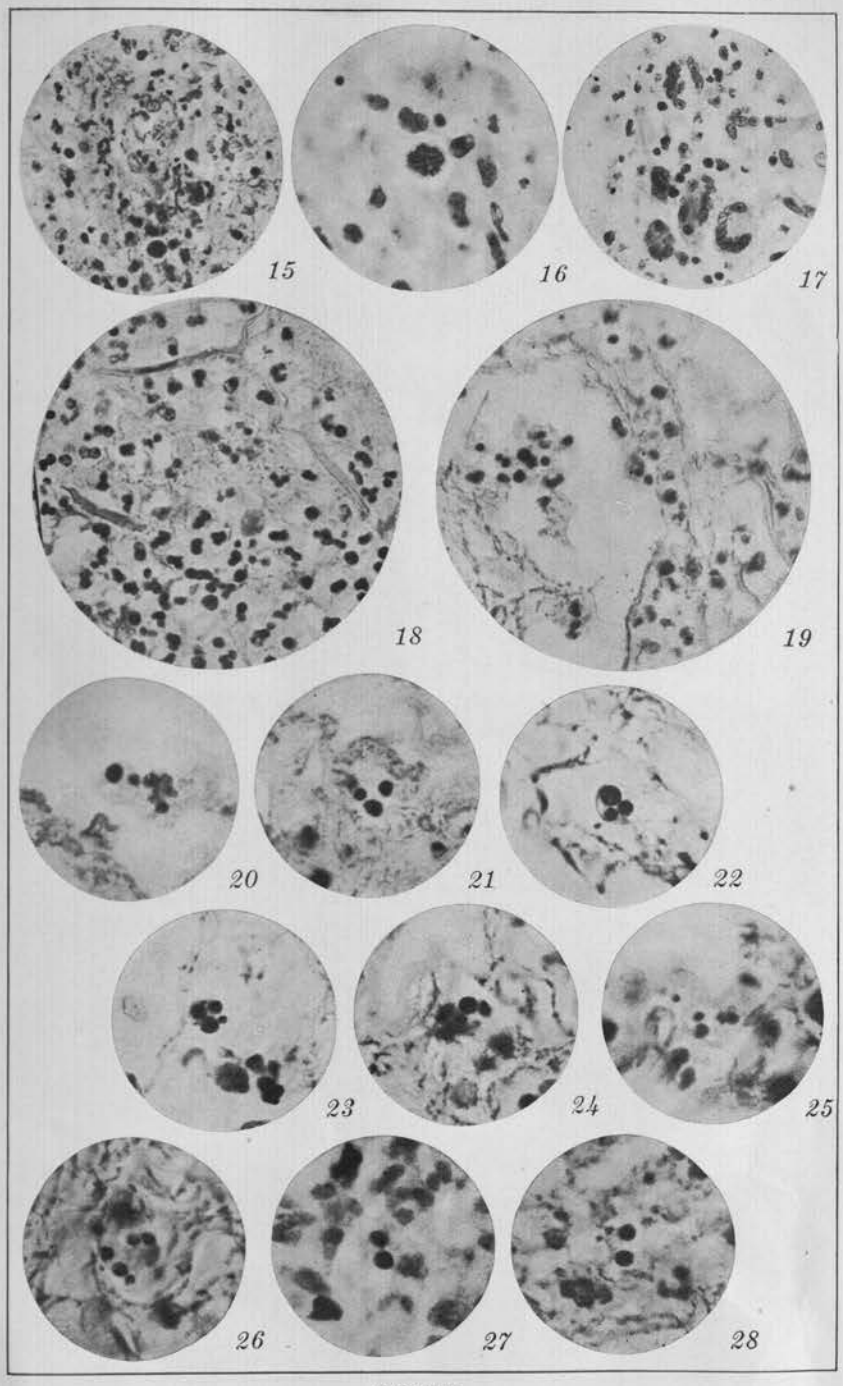

PLATE III.

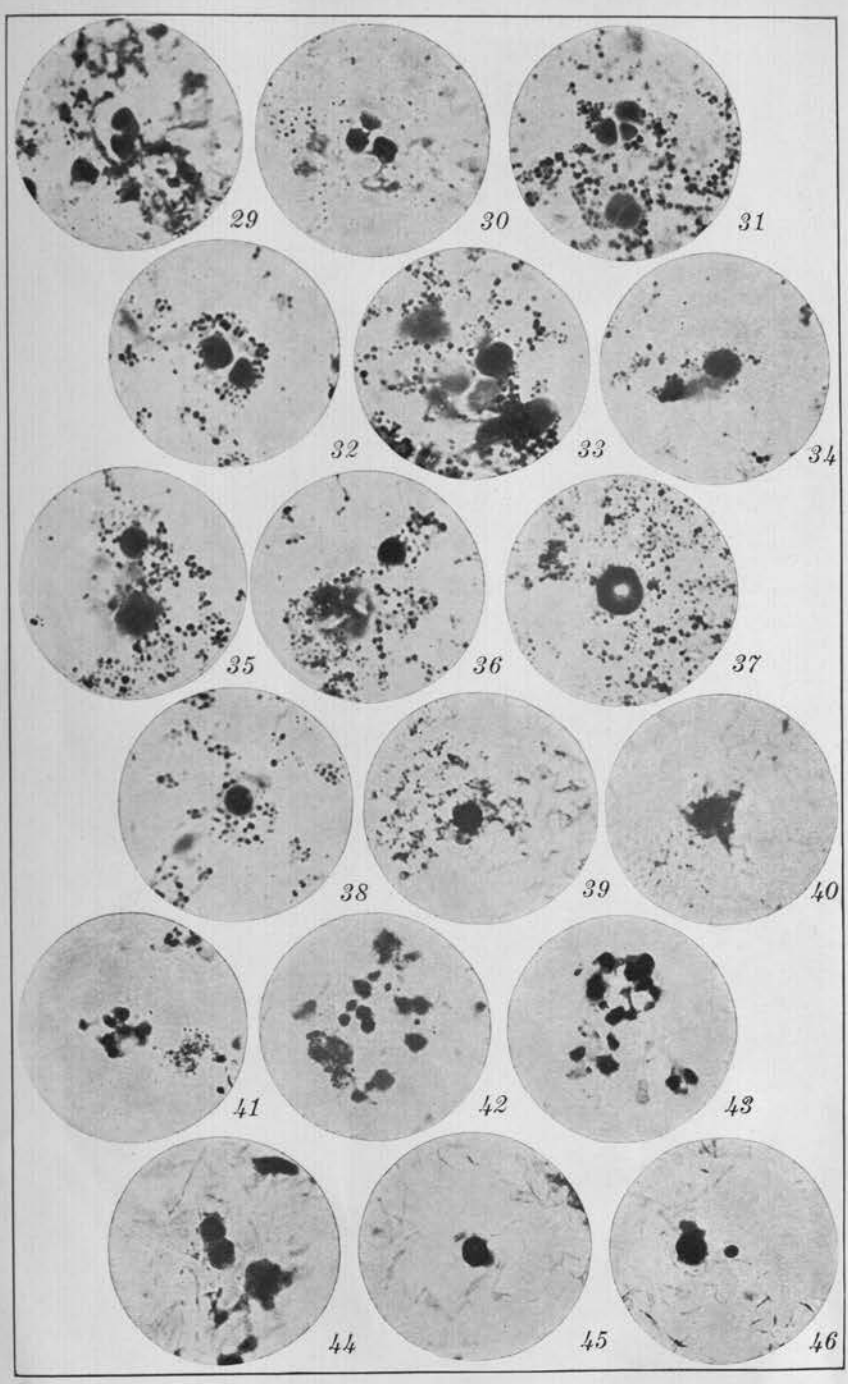

PLATE IV.

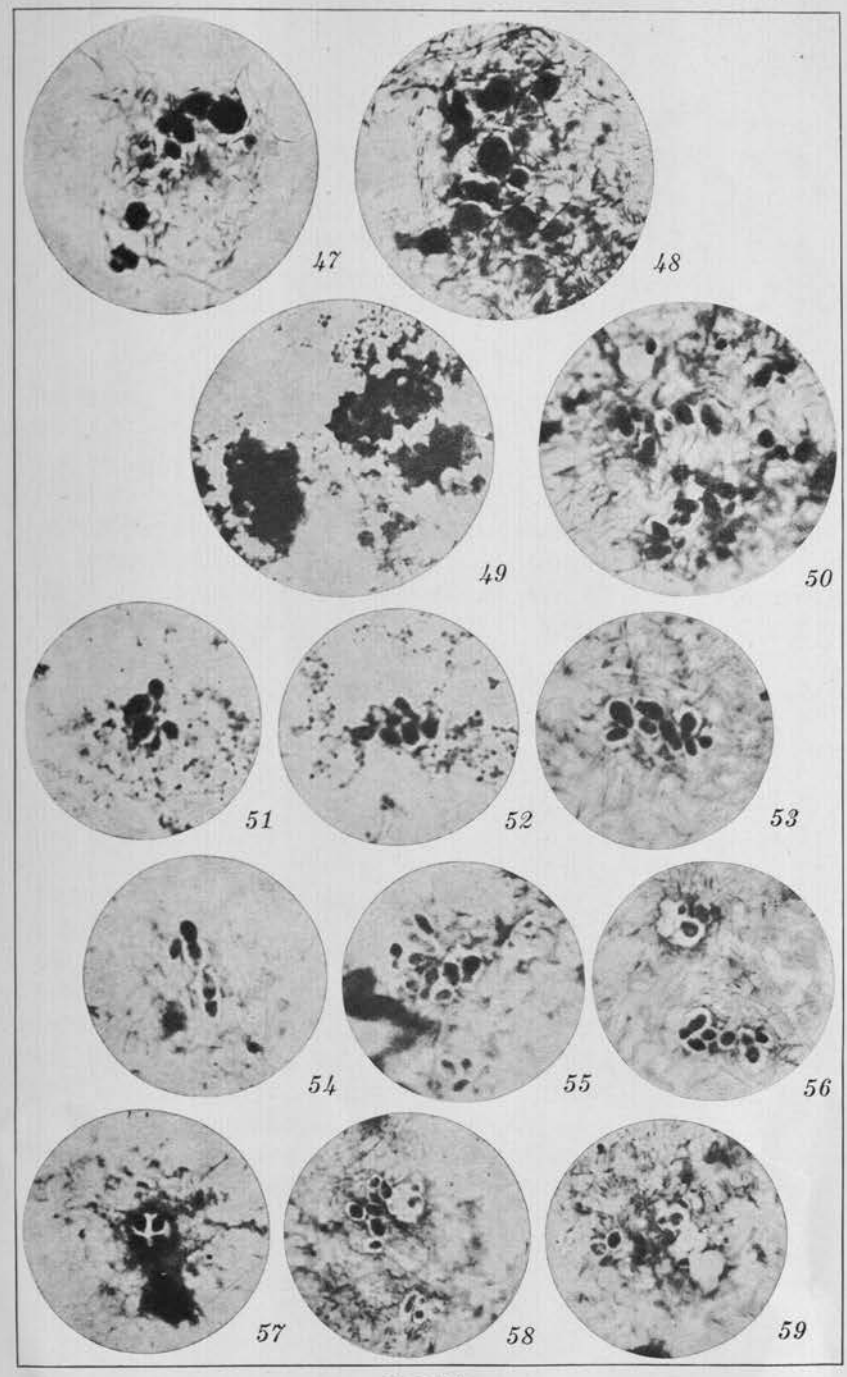

PLATE V.

FURTHER OBSERVATIONS ON THE TREATMENT OF YAWS WITH CASTELLANI'S MIXTURE

By L. E. GUERRERO, E. DOMINGO, and M. ARGÜELLES

(From the Department of Medicine, College of Medicine and Surgery, University of the Philippines)

TWO PLATES

Salvarsan and neosalvarsan are, without doubt, the specific remedies for frambœsia; but the high price and shortage of these drugs brought about by the war, the lack of hospital facilities in districts where the disease prevails, and the fact that patients often refuse any kind of injection treatment are some of the considerations that led Castellani,(1, 2) in 1915, in Ceylon, and us in the following experiments to use his formula in place of the drugs. The formula consists of:

	Quantity.
Tartar emetic ..... grams..	0.065
Sodium salicylate ..... do....	0.65
Potassium iodide ..... do....	4
Sodium bicarbonate ..... do....	1
Water ..... do....	30

The above is given in one dose, diluted in 4 ounces of water, thrice daily, for adults and for children over 14 years of age, half doses to children 8 to 14 years of age, one-third doses or less to younger children, and not more than half doses to Europeans. According to Castellani the preparations of antimony, which were first introduced in the treatment of yaws by Brault in 1911, have a beneficial effect, but their action is slow. Sodium salicylate apparently hastens the disappearance of the thick yellow crusts, due to secondary pyogenic infections. Potassium iodide potentiates tartar emetic, but its great drawback is that the good effects are obtained only by using doses that often give rise to iodism. This and the emetic action of the antimony and potassium tartrate are diminished by the addition of sodium bicarbonate. The formula is pharmaceutically a very inelegant one; it is cloudy and has a sediment due to the formation of antimony

oxide. The sediment, however, disappears when each dose is diluted with four times the amount of water or when 8 cubic centimeters of glycerin are added to the formula.

Castellani tried this formula in eleven cases given in the doses mentioned for from ten to fifteen days, followed by from five to ten days of rest, then another course for another five or ten or fifteen days. His results were very satisfactory in recent and fairly recent cases, in which the disease had started three to twelve months previously. In chronic cases the results were much less satisfactory. Very mild symptoms of iodism were observed in three cases, but were not sufficiently severe to necessitate stopping the treatment or decreasing the doses. No symptoms pointing to any depressing action on the heart were noted.

Spaar(5) obtained excellent results with this treatment in three cases, the patients recovering in two or three weeks.

We have used this treatment in more than 43 cases, including 7 in the Philippine General Hospital, 14 in Parañaque, and 22 in Las Piñas. The cases in the Philippine General Hospital were all discharged completely cured.

With the exception of four, all our cases presented, more or less moderately, one or several of the following symptoms during the treatment: General malaise, weakness, slight fever, nausea, vomiting, gastralgia, diarrhœa, pharyngitis, ptyalism, intense coryza, lachrymation, congestion of the conjunctiva, cephalalgia, and insomnia. One case also presented slight induration or nodules of the skin of the face and ears, and another presented erythematous patches in various parts of the body. However, we did not observe in those cases who were under our immediate supervision furunculosis, acne, purpura, nor œdema of the eyelids, face, and larynx. The method of administration followed by us brings about gradual saturation of the body. We gave on the first day one-third dose three times; on the second day, one dose twice; and on the subsequent days, one dose three times daily. We adopted this dosage in order to ascertain the susceptibility and gradually to establish tolerance in the patient. The number of doses necessary to effect a cure varied from 15 to 80, and the time elapsing from the commencement of the treatment till complete recovery was from five to twenty-seven days.

Of the 14 cases of Parañaque, 10 showed complete recovery, while the remaining 4 showed only improvement of the symptoms. Seven of the Las Piñas cases discontinued the treatment early, 3 showed improvement of symptoms, 3 proved refractory, and 9 recovered completely. To summarize: Of the 36 cases that continued the treatment, 24 completely recovered, 7 showed im-

provement of symptoms, 7 showed no improvement at all, and 5 had relapses in from two to five months after the lesions had entirely healed.

The details of the cases treated and the results of the treatment are given in the subjoined protocols:

Case I.—M. P., female, 45 years old, a native of Bulacan. The duration of the infection was about nine months. The lesions consisted mainly of granulomatous ulcers on the labia majora and a few papillomatous growths about the nasal fossæ and the left angle of the mouth. The growth was covered with a thick yellowish crust. The ulcerations on both labia excreted a purulent fetid material. The general appearance of the growth simulated "granuloma pudendi." The patient received 44 doses of Castellani's mixture in nineteen days, and sixteen days later the lesions were completely healed.

Case II.—F. P., female, 24 years old. The infection was of six months' duration. The lesions consisted of warty growths on the forehead, face, neck, abdomen, and genitals. The mother yaw was an ulcer with irregular borders, 2 centimeters in diameter, on the anterior surface of the left ankle. The patient entered the hospital to obtain relief from pain in the bones and joints. The joints of the fingers and hands were swollen and painful. She received 23 doses in ten days and left the hospital after fifteen days with complete disappearance of arthritic pain and cicatrization of the lesions.

Case III.—C. F., male, 30 years, married, denied having had venereal diseases. The duration of the infection was one year and four months. The patient thought that he had contracted the disease from his children, who had yaws. The primary lesion appeared as a bed bug bite on the left thigh. On scratching the skin over it, the lesion gradually increased in size to about 10 centimeters in diameter. Two months later eruptions appeared all over the body, but after a year they disappeared without treatment. Last January he was admitted to the hospital, complaining of pain in the bones. He presented extensive ulcerations on the soles of both feet. The Wassermann test was negative. The patient was given 15 doses in eleven days. He left the hospital completely cured.

Case IV.—E. Y., female, 19 years old, contracted yaws six months ago. She said she had had yaws when she was 12 years old. The scars were plainly visible on the face and on other parts of the body. She had an ulcer on the anterior aspect of the right leg, which measured 3 centimeters in diameter and 1 centimeter deep. The edges were irregular, and the base was formed by necrotic tissue and exposed bone. Wassermann reaction was positive. The patient was given 5 drops of saturated solution of potassium iodide and 5 milligrams of protoiodide of mercury three times daily. Aseptic dressing was applied to the ulcer. She experienced no improvement from this treatment. Castellani's formula was then given three times daily, and 25 per cent protargol ointment was applied locally. After 54 doses the ulcer completely healed, although the pain in the bones persisted for some time.

Case V.—M. Santa Ana, male, 38 years old. The duration of the illness was five months. The lesions consisted of granulomatous growths of varying size, whose summits were covered with yellow crusts. They were distributed on the scalp, forehead, chin, neck, shoulder, back, and buttocks. The lesions appeared in crops, accompanied by severe pain in the bones and

swelling of all the joints. The bones of the fingers were markedly enlarged. He took 24 doses, and after nine days in the hospital the symptoms disappeared and the lesions healed.

Case VI.—S. P., 17 years old, male, had yaws for a month. There were papules covered with a yellowish crust in the right nostril, and there were also scars. The patient entered the hospital on account of pain in the bones and swelling in the joints. He said he had had a similar attack one year ago. He took 14 doses in six days. The treatment caused amelioration of the pain and of the lesions.

Case VII.—A. M., male, 40 years old, contracted yaws four years ago. He came for treatment of an old ulcer on the right forearm. It measured about 4 centimeters in diameter. The edges were irregular and elevated, and the base was covered with yellowish purulent material. The patient stayed in the hospital twenty-seven days, during which time he took 80 doses. The ulcer had completely healed when he was discharged from the hospital.

Case VIII.—L. S., male, 31 years old, had yaws for one year and four months. The lesions were granulomatous nodules of from 1 to 1.5 centimeters in diameter. They were found on the neck and chest. The face showed numerous pigmented scars. The patient was given 10 drops of a saturated solution of potassium iodide three times daily for nine months. The iodide produced only insignificant improvement of the eruptions on the face. Castellani's mixture was then given, and in five days the lesions completely healed.

Case IX.—F. C., female, 40 years old. The patient said she had had an eruption eight years ago. She came for treatment of an ulcer on the right external malleolus. The ulcer was of five months' duration. It was about 4 centimeters in diameter and was covered with fetid whitish material; the border was elevated. The ulcer completely healed after the patient had taken 39 doses in a period of fifteen days.

Case X.—F. de G., 40 years old, male, had yaws for one year. The lesion appeared as an abrasion of the skin of the forehead. It later developed into an ulcer. There were four ulcers on the forehead, from 5 to 10 centimeters in diameter, at the time of treatment. The edges were elevated and granular. Castellani's treatment caused complete cicatrization of the ulcers.

Case XI.—C. S., female, 16 years old, was ill for four months. At first she had a febrile pain in the joint and itching of the skin over the left knee. The skin then became covered with a yellow crust, which she thought was scabies. Later small papules appeared around the lesion on the knee. These spread in two weeks all over the body and assumed the characteristic appearance of the manifestation of yaws. The patient believed she had contracted the disease from a visitor who had the same disease. She took 47 doses in eighteen days, and the lesions completely disappeared.

Case XII.—J. F., 34 years old, was the mother of case XI. She had yaws for three months. The lesions consisted of granulomatous papules on the face and hands and of scaly eruptions on the forearm. The latter were arranged in the form of islands 3 to 4 centimeters in diameter. She was admitted with case XI. After 41 doses, given in sixteen days, she recovered completely.

Case XIII.—J. T., 27 years old, married, female, had yaws for three months. The mother yaw was found on the left middle finger. Seven days later granulomatous papules appeared on the face and other parts of the

body. She complained of pain in the bones and joints, but no swelling was found. She stayed in the hospital twenty-three days and took 66 doses. When she was discharged, all the eruptions had completely healed. The last three cases were relatives living in one house. Their infection seems to originate from the visitor mentioned in case XI.

Case XIV.—F. O., male, 8 years old. He developed secondary yaws nine months ago. On examination there were found secondary lesions, especially on the penis and about the anus, and an ulcer with granulomatous, irregular border on the left internal malleolus. The diagnosis was tertiary yaws. Thirty doses produced complete cicatrization of the ulcers.

Case XV.—E. C., female, 42 years old, had yaws in 1898. At the time of the examination she presented an ulcer on the posterior surface of the right forearm below the elbow. The surface of the ulcer was granular and was covered with fetid, purulent grayish secretion. It measured about 8 centimeters in its longest, and 5 centimeters in its shortest, diameter. It began as an induration of about 0.5 centimeter. It gradually increased in diameter to about 3 centimeters. The diagnosis was tertiary yaws. The ulcer healed without local treatment after the patient had received 30 doses of Castellani's mixture.

Case XVI.—I. P., female, 30 years old, had had yaws at the age of 7 years. About three years ago she was hit on the right knee. A nodule appeared on this knee. Later it became an ulcer with deep, irregular borders and fetid yellowish gray secretion. The joint was swollen and painful, incapacitating the patient for walking. The diagnosis was tertiary yaws. She was given 36 doses of Castellani's mixture, and the ulcer completely healed.

The treatment of Castellani undoubtedly exerts a curative influence on the various manifestations of framboesia. In six or seven days of treatment the granuloma takes on a livid appearance, becomes surrounded by a pinkish halo, and begins to flatten. The crust dries and gradually disappears, leaving a macule that eventually disappears. In deep and extensive ulcers cicatrization takes place gradually and concentrically, the entire process lasting from fifteen to thirty days. Pain in the bones, pain and swelling of the joints, and pruritus are relieved early in the treatment, although sometimes the pain in the bones and joints recurs even after complete healing of the lesions.

The relapses and incomplete recovery observed in some cases are to be ascribed to an incomplete medication, due to the suspension of treatment before the destruction of *Spirochæta pertenuis* was complete. Some of our cases discontinued the treatment after the disappearance of granulomata and ulcers; while others, who were not under our direct supervision, could take only a few doses irregularly. We were not able to supply the patients with the adequate amount of the medicine, because the experiments were carried out in neighboring towns, where we had to distribute the preparation gratis. On the whole, the

patients do not seem to object to this treatment; on the contrary, they willingly submit themselves after they are convinced of its gratifying results.

Our results confirm the conclusion of Castellani that the diverse manifestations of frambœsia heal under the influence of his treatment. The cure of recent infections by this preparation is nearly as marvelous as that by salvarsan and neosalvarsan. We cannot assert as yet whether or not the cure is permanent, since only a limited number of cases remained under our observation for a long time. We believe, however, that the continuation of the treatment after the lesions have healed (from five to ten days' treatment with intermissions of from ten to fifteen days) will insure a permanent cure.

The tendency of the mixture to cause emesis and iodism is not a serious objection to its routine employment, because these untoward effects are slight and may be easily corrected. If emesis, gastralgia, and diarrhœa are troublesome, they can be prevented by merely increasing the sodium bicarbonate or by giving, fifteen minutes before each dose, 4 cubic centimeters of paregoric or 1 centigram of codeine. Other untoward effects, which are apparently due to vasomotor disturbances, such as œdema, lachrymation, coryza, congestion of the conjunctiva, etc., are readily overcome by epinephrine. According to Milian, (3, 4) this drug also gives excellent results in iodism and cerebral symptoms associated with the use of arsenobenzol. He strongly advocates the administration of massive doses of the drug, for example, 3 milligrams per orem twice daily, and if this fails, 1 or 2 milligrams injected subcutaneously or intramuscularly.

In the administration of Castellani's mixture it is always important to bear in mind the possible presence of the larval form of hyperthyroidism and also the greater susceptibility of children to iodides, for large doses may give rise to alarming and very unpleasant side effects.

Frambœsia, though not a grave malady quod vitam, has a tendency to relapse, running a highly chronic course extending for years, and gives rise to rheumatoid pains, osseous deformities, extensive ulceration (which may serve as a portal of secondary infection), and deforming cicatrices, which not only are disfiguring but often more or less seriously incapacitate the patients, school children and adults alike. Admitting that it is not a source of high mortality, it is, however, decidedly a cause of high morbidity, entailing material loss to the individual and to the community.

Frambœsia is one of the common diseases in the Philippine Islands and is widely distributed. It is impossible to estimate the extent of infection, but it is undoubtedly of considerable importance. The establishment of ambulatory or provisional hospitals in the larger infested districts for the adequate administration of salvarsan would be the ideal method of suppressing this disease. This being impossible under present conditions, the general use of Castellani's treatment, under suitable administration, offers a practicable means of combating the disease.

We desire to express our gratitude to Doctors Calderon, Lantin, and Reyes and to Messrs. Hallare and Fernandez for cooperation that made this work possible.

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ILLUSTRATIONS

PLATE I. YAWS

FIG. 1. Case VIII, before treatment.
2. Case VIII, five days after treatment.
3. Case XIII, before treatment.
4. Case XIII, after 41 doses given in sixteen days.

PLATE II. YAWS

FIG. 1. Case XV, before treatment.
2. Case XV, after 30 doses of the mixture.
3. Case XVI, before treatment.
4. Case XVI, after 36 doses of the mixture.

Fig. 1. Case VIII, before treatment.

Fig. 2. Case VIII, five days after treatment.

Fig. 3. Case XIII, before treatment.

Fig. 4. Case XIII, after 41 doses given in sixteen days.

PLATE I. YAWS.

Fig. 1. Case XV, before treatment.

Fig. 2. Case XV, after 30 doses of the mixture.

Fig. 3. Case XVI, before treatment.

Fig. 4. Case XVI, after 36 doses of the mixture.

PLATE II. YAWS.

RECONSTRUCTION OF TWO SETS OF DUCK TWINS, WITH SPECIAL REFERENCE TO THE EARLY EMBRYONIC DEVELOPMENT OF THE VASCULAR SYSTEM [1]

By EDWARD S. RUTH

(*From the Department of Anatomy, University of the Philippines*)

TWO PLATES

The early stages of a large series of chick and duck twin embryos have been studied especially by Kaestner, (2) and a few have been recorded by Burckhart, (1) Reichert, (4) and Mitchell. (3) However, up to the present time only a few of the embryonic twins have been reconstructed. Kaestner apparently was the pioneer in making reconstructions of young twin embryos. Those figured by him are considerably older than the duck twins under discussion in this paper. Much may be elucidated regarding the early formation and intimate relation of embryonic twins by careful detailed study of the serial sections and especially by making a reconstruction. In a previous communication (5) I described four sets of duck twins. Set III of that paper has been reconstructed and is figured in this contribution; set V was found subsequent to the issue of the previous publication. These two sets of duck twins have been reconstructed according to the Borne method. I wish to express my appreciation and thanks to Dr. Ricardo Molina for the reconstruction of duck twins set V.

DUCK TWINS SET V

The duck twins are in the 9-somite stage of development. The head and thoracic regions lie parallel to each other, while the caudal extremities diverge, forming an angle of about 55°. Surrounding the embryos is the area pellucida, which is transparent. It is more marked on the ventral side of the head in the region of the proamnion.

Ventral side.—The heads are abnormally large and somewhat club-shaped. In the region of the fovea cardiaca the membranes have an increased opacity, extending laterad to the

[1] Received for publication October 13, 1917.

embryos and pointing caudad. The former is apparently the position of the omphalomesaraic, and the latter is apparently the position of the median vein. The posterior openings of the foregut are separated from each other by a rounded, narrow partition. On cross section a tubular vessel occupies this space in the partition, separating the posterior ends of the foregut. At the extreme tip of the caudal extremity, remains of the primitive streak are still present.

Dorsal side.—The heads project slightly dorsad to the proamnion. They are distinctly separated from each other for a short distance where the ectoderm becomes continuous from one head to the other. On the median side of the heads there is a small cleft where the medullary groove has failed to close entirely. This marks the position of the anterior neuropore. The medullary groove has closed over in the cephalic region, caudad to the anterior neuropore in both embryos; however, it again becomes open and remains so throughout its entire extent. The caudal portion of the medullary groove in the right embryo is slightly more expanded than in the left. The somites are regularly developed. The medial ones are somewhat larger than those on the lateral sides of the embryos.

Reconstruction of duck twins set V.—This set of twins is somewhat younger than set III. The foreguts are symmetrically developed, the openings of which are at first common to both components, but they are soon divided and separated from each other by a fold of mesoderm. The tubular hearts are indistinctly developed and appear to be double at the middle and caudal third. The omphalomesaraic veins join the tubular hearts, the left vein having a position much more cephalad than the one on the right side. Vaso formative cells link the one tubular heart with the other. On the dorsal side several median veins can be seen lying on the medial anterior wall between the two pharynges. The venous confluence can be made out rather indefinitely; it is not so distinct as in set III. As the single tubes approach the cephalic end of the pharynx, the one on the left component turns abruptly laterally and downward to gain the dorsal side of the pharynx, where it becomes the dorsal aorta. The tubular heart in the right component passes obliquely and laterally over the most cephalic portion of the pharynx to gain the posterior side. Both dorsal aortæ pass caudad along the lateral and dorsal side of the pharynx and are lost in the area pellucida after leaving the embryos. On the dorsal side of the pharynx some distance above the opening of the foregut a median dorsal vein also makes

its appearance (Plate II). This, like the other, is also lost in the interembryonic tissue at a distance slightly more caudad.

DUCK TWINS SET III

Reconstruction of duck twins set III shows the foreguts and vascular system symmetrically developed in every detail (Plate II). In the region of the thorax, abdomen, and caudal extremity the ectoderm and entoderm are continued from one embryo to the other. The dorsal aortæ are all distinctly and uniformly developed, though their continuation into the vitalline arteries cannot be traced. In the region of the fovea cardiaca the venous blood vessels form a median confluence by the union of the two omphalomesaraic veins with the median vein, which approaches the embryos from a caudal interembryonic direction. The large median vein is formed from a series of smaller veins beginning symmetrically from eight smaller veins. Extending cephalad to the venous confluence are two tubular projections. The tubular hearts are symmetrically developed. Both hearts, as they approach the cephalic portion of the foreguts, divide into two ventral aortæ, which loop around the lateral sides of the pharynx to form the dorsal aortæ. The dorsal aortæ terminate rather abruptly in the region of the venous confluence on the posterior side of the foregut. No vitelline arteries appear to be present. The medullary tubes and grooves are entirely separate and distinct from each other throughout the entire extent of the embryos; both are symmetrically developed. On the ventral side of each medullary tube is a notochord. The foreguts are uniform in every detail. Each component in this set of twins is separately developed, with the exception of the venous confluence. Here the two embryos appear to have a vascular chamber in common (Plate II). All other structures at this stage of development are duplicated.

DISCUSSION

The two sets of twins have a number of developmental parts in common. However, in set V the medial dorsal arteries are only partially developed, while the lateral have already joined with the ventral aortæ. In the older twins the blood vessels have apparently assumed a fixed position in relation to the embryo, while in the younger twins they are in a migrating stage, that is, in the younger twins the heart anlage has not yet become tubular in several areas. In both twins there is present a venous confluence at the proximal end of the tubular hearts formed by two omphalomesaraic veins and a median vein.

Kaestner shows a similar confluence in a set of his chick twins, but does not show a median vein. Furthermore he considers the confluence in the process of developing into a single heart and concludes that in all probability if growth had continued there would have developed a single heart. The confluence of the veins does not correspond to the position of the heart in embryos of this stage of development, but from the single tubular heart that lies more cephalad. If any conclusion can be drawn, one heart should develop from each tubular heart, as this corresponds to the region where it normally develops.

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ILLUSTRATIONS

[Drawings by J. Castro.]

PLATE I

FIG. 1. Ventral view of duck twins V. *pa*, proamnion; *fc*, fovea cardiaca; *ls*, lateral somite; *cd'*, *cd*, chorda dorsalis; *ps*, primitive streak.

2. Dorsal view of duck twins V. *pa*, proamnion; *an*, anterior neuropore; *mg'*, *mg*, medullary groove; *ls*, lateral somite; *mv*, median veins; *ap*, area pellucida.

PLATE II

FIG. 1. Dorsal view of pharynx and aortæ from duck twins III. *ph'*, *ph*, pharynx; *da'*, *da*, dorsal aortæ; *omv'*, *omv*, omphalomesaraic vein; *mv*, median vein; *veh*, venous end of heart.

2. Ventral view of pharynx, tubular hearts, and aortæ from duck twins III. *ph'*, *ph*, pharynx; *th*, tubular heart; *ve*, ventral end of heart; *va*, ventral aorta; *omv*, omphalomesaraic veins; *mv*, median vein.

3. Ventral view of pharynx and aortæ, duck twins V. *omv'*, *omv*, omphalomesaraic vein; *ph*, pharynx; *th'*, *th*, tubular heart; *of*, opening of foregut; *mv*, median vein.

4. Dorsal view of pharynx and aortæ, duck twins V. *lda'*, *lda*, lateral dorsal aortæ; *ph*, pharynx; *mda*, median dorsal aortæ; *mv*, median vein.

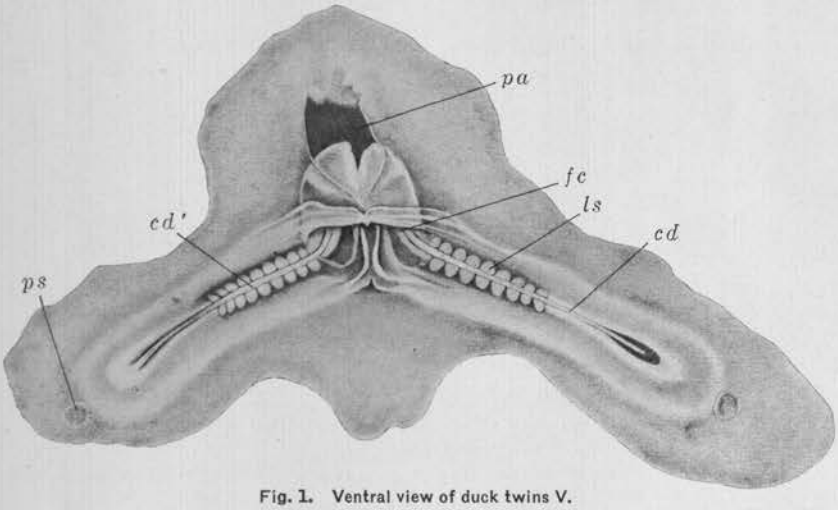

Fig. 1. Ventral view of duck twins V.

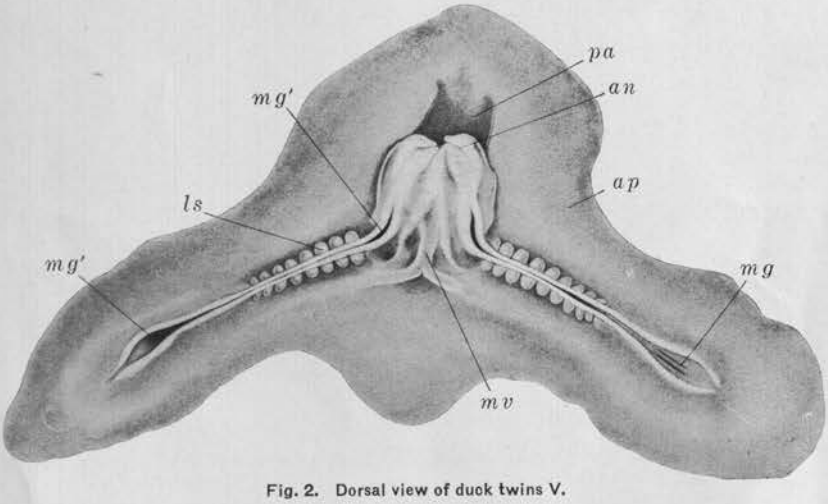

Fig. 2. Dorsal view of duck twins V.

PLATE I.

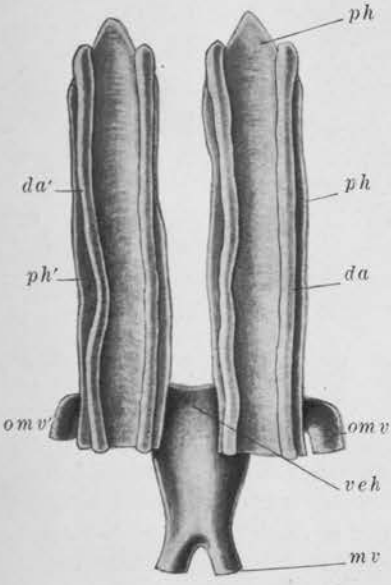

Fig. 1. Dorsal view of pharynx and aortæ. Duck twins III.

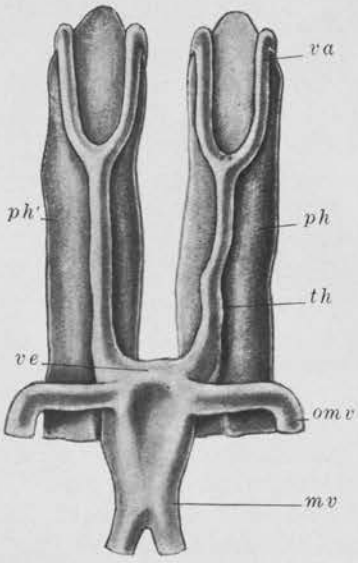

Fig. 2. Ventral view of pharynx, tubular heart, and aortæ. Duck twins III.

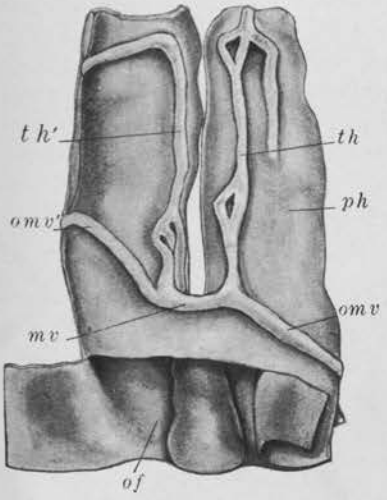

Fig. 3. Ventral view of pharynx and aortæ. Duck twins V.

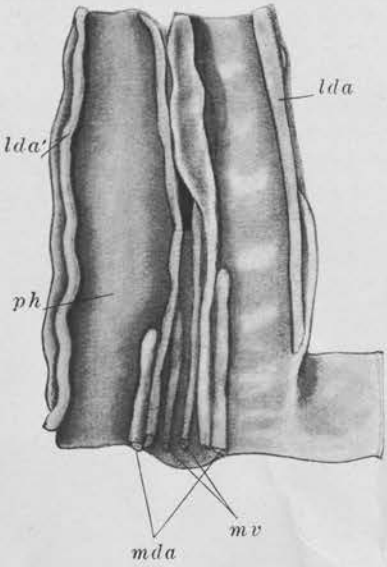

Fig. 4. Dorsal view of pharynx and aortæ. Duck twins V.

PLATE II.

REVIEWS

The Prescription | therapeutically, parmaceutically, grammatically and | historically considered | by | Otto A. Wall, Ph. G., M. D. | [7 lines] | fourth and revised edition | St. Louis | C. V. Mosby Company | 1917 | Cloth, pp. 1–274. Price, $2.50.

An extremely interesting and readable book, but not one commending itself as a textbook. The desire of the publishers to present a typographically perfect book has apparently been well carried out. The use of the term "yelk" for yolk of egg on page 229 is very questionable.

J. A. J.

Volume I Johns Hopkins Number | Number I | The | Medical Clinics | of | North America | July, 1917 | Published bi-monthly by | W. B. Saunders Company | Philadelphia and London | [6 lines]. Pp. 1–193. Price, per year: Paper, $10 (foreign, £2 2s); cloth, $14.

The first number of The Medical Clinics of North America gives one a favorable impression. The feature in this publication is the question and answer portion by which means the lecturer's ideas on diagnosis and treatment are forcibly brought before the reader.

J. A. J.

Issued with THE PHILIPPINE JOURNAL OF SCIENCE, SECTION B,
Tropical Medicine, Vol. XIII, No. 4, July, 1918.

PROCEEDINGS OF THE MANILA MEDICAL SOCIETY

REGULAR MONTHLY AND ANNUAL MEETING, JANUARY 7, 1918

MINUTES OF THE MANILA MEDICAL SOCIETY

The regular monthly and annual meeting of the Manila Medical Society was held in the College of Medicine and Surgery on Monday evening, January 7, 1918, at 8.30, with President Ruth presiding. There were 18 members and 2 visitors present.

The minutes of the last meeting were read and approved as read.

The applications for active membership of Drs. A. Francis Coutant, Emiliano M. Panis, and Isidoro de Santos were presented with the recommendation of the council. It was moved and seconded that the secretary be instructed to cast a unanimous ballot in favor of the above applicants. The motion was carried.

The program for the evening was then carried out as follows:

1. Glioma Retinæ, by Dr. Herminio E. Velarde.
2. Poisonous Snakes of the Philippines, by Mr. E. H. Taylor.
3. A Report of Five Cases of Leukemias Clinically Diagnosed as Splenomegaly, by Dr. José S. Hilario. (This paper was read by title.)

The annual report of the secretary-treasurer for 1917 was read and approved as read.

The president next appointed Drs. B. C. Crowell, R. B. Gibson, and José Albert as a committee on nominations for the officers of the society for the ensuing year. The meeting was suspended for a few minutes pending the return of the nominating committee.

The nominating committee then returned and recommended the following:

For President:	Dr. Otto Schöbl.
For Vice-President:	Dr. H. G. Maul.
For Secretary-Treasurer:	Dr. Daniel de la Paz.
For Councilor for three years to succeed Dr. J. E. Reed, Jr.:	Dr. E. S. Ruth.
For Councilor for five years:	Dr. A. P. Goff.

There being no objection to the nominations, it was moved, seconded, and carried that the election of the above-named officers as recommended by the committee on nomination be approved.

The newly elected officers were then informally installed, and in the absence of President Schöbl, Vice-president Maul presided.

On motion duly made and seconded, Doctor Gibson was unanimously elected editor of the proceedings, and a vote of thanks by the society was extended him for his excellent and proficient work in the past year.

A vote of thanks was also extended to the outgoing officers.

There being no further business, it was announced that there would be no meeting in February, because of the joint meeting of the Philippine Islands Medical Association and the IV Asamblea Regional de Médicos y Farmacéuticos de Filipinas, and the meeting adjourned.

H. G. MAUL,
Secretary-Treasurer.

SCIENTIFIC PROGRAM

SIX CAŞES OF GLIOMA RETINÆ

By DR. HERMINIO E. VELARDE

Case histories, illustrated with photographs and skiagrams, of six cases of glioma retinæ were presented. All six cases were females, the ages being from 1¾ to 4 years; the condition in one case was observed shortly after birth. Cases reported in the literature have occurred in children from 10 weeks to 13 years of age. Other members of the families were not affected. In two out of the six cases the condition was bilateral; the usual figure is 25 per cent of bilateral cases. Metastatic involvement of the liver was observed in two cases, of the scalp in two cases, and also of the cervical glands, ribs, shoulders, thighs, pleura, and dura mater. The prognosis of the disease is serious and almost certainly fatal, as exemplified by the present series, if early enucleation is not done.

POISONOUS SNAKES OF THE PHILIPPINES

By MR. E. H. TAYLOR

Of the 25 deadly poisonous snakes known in the Islands, 18 species were shown in the demonstration. Of the Crotalinæ, or pit vipers, the following were examined: *Trimeresurus gramineus*, from Luzon; *T. flavomaculatus*, from Luzon; *T. schultzei*, from Palawan; *T. wagleri* (and two varieties), from Mindoro and Palawan; *T. haliens*, a rare species from Polillo.

A new yellow viper (recently discovered on Batan Island, Batanes Islands), as yet undescribed, was shown.

The poison apparatus of this group was discussed, and the large fangs, which attain a greater size in this group than in any other, were observed and discussed.

Of the Elapidæ, which are the most deadly of reptiles, good specimens of the following were shown: *Naja hannah*, the king cobra, or hamadryad; *N. naja caeca*, from Luzon; *N. n. miolepis*, from Palawan; and *N. n. samarensis*, from Mindanao.

The last three are the Philippine representatives of *Naja naja tripudians*, the famous spectacled cobra of southern Asia. These species are known in Luzon as *alopong*, or *carasaen*, the latter name being used in the north in the Ilocos provinces and Pangasinan. They may be readily recognized, when alive, by the habit of spreading the neck; and in the three subspecies they may be recognized by the loud hissing noise made. They are able to throw the poison to a distance of at least two meters.

Of the related genera, specimens of *Hemibungaris* and *Doliophis* were displayed. Special attention was called to the unique poison glands found in the latter genus. Instead of being situated in the head, they form long club-shaped glands, which are contained in the body cavity; these attain a length in adults of at least 20 centimeters.

Specimens of the aquatic genera *Disteira*, *Lapemis*, and *Laticauda* were shown. These are the sea snakes; they occur in large numbers in Manila Bay.

The Opisthoglypha (poisonous snakes not regarded as deadly) were represented by several species of *Boiga* and by the color varieties of *Dryophis prasinus*, the widely famed *dahon palay*.

A few harmless forms were discussed that approximate the poisonous forms in color, markings, and habits, and so are very frequently confused with them.

It was requested that more careful observations be made and records be kept by provincial doctors of cases of snake bites, that effort be made for a more careful recording of statistics of death from snake bites by health authorities, and that antivenin serums be manufactured in the Islands.

R. B. GIBSON,
Editor of the Proceedings,
Manila Medical Society.

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Tropical Medicine, Vol. XIII, No. 4, July, 1918.

PROCEEDINGS OF THE MANILA MEDICAL SOCIETY

REGULAR MONTHLY MEETING, MARCH 4, 1918

MINUTES OF THE MANILA MEDICAL SOCIETY

The regular meeting of the Manila Medical Society was held at the College of Medicine and Surgery, March 4, 1918, at 8.30 in the evening.

The following members were present:

Dr. B. C. Crowell.	Professor F. G. Haughwout.
Dr. E. S. Ruth.	Dr. H. W. Wade.
Dr. F. W. Vincent.	Dr. J. S. Hilario.
Dr. R. B. Gibson.	Dr. F. Garcia.
Dr. B. Samson.	Dr. D. de la Paz.

The secretary announced the absence of the president and vice-president and with the consent of the society appointed Dr. B. C. Crowell to officiate as temporary chairman.

The minutes of the previous meeting were read and approved.

A letter from the President-elect, Dr. Otto Schöbl, declining the acceptance of the presidency of the society, was read and accepted with regret.

Doctor Crowell appointed Doctors Ruth, Gibson, and Samson to constitute a committee on the nomination for the president.

The society took a short recess.

At the second session the committee announced that Dr. F. W. Vincent was unanimously nominated. There being no other nominations, the secretary was instructed to cast a unanimous ballot in favor of the nomination.

With President Vincent in the chair, the reading of the papers was then proceeded with.

The first paper, entitled Studies of Cerebrospinal Fluid in Acute Anterior Poliomyelitis, by John A. Kolmer and co-workers, abstracted by Dr. John A. Johnston, was read by Doctor Gibson in the absence of Doctor Johnston.

Doctor Wade presented his paper, entitled Cryptoplasmic Infection, which was discussed by Professor Haughwout and Doctor Crowell, Doctor Wade closing the discussion.

The last paper, entitled The Prophylaxis of Malaria, was read

by its author, Doctor Samson. It was discussed by Professor Haughwout.

The society adjourned at 9.55 in the evening.

D. DE LA PAZ,
Secretary-Treasurer.

SCIENTIFIC PROGRAM

STUDIES OF THE CEREBROSPINAL FLUID IN ACUTE ANTERIOR POLIOMYELITIS[1]

By JOHN A. KOLMER and co-workers
Abstracted by DR. JOHN A. JOHNSTON

The results summarized in this paper are based upon the examinations made in 868 specimens of cerebrospinal fluid obtained during the epidemic in Philadelphia during 1916.

A summary follows:

1. The majority of the fluids were water clear or but faintly opalescent when viewed against a black background.

2. Seventy-seven per cent of the fluids showed an increase of total cells.

3. In over 96 per cent of the fluids from cases after the onset of paralysis the small lymphocyte variety of cells predominated. Polymorphonuclear cells predominated in less than 1 per cent of the fluids, and in over 88 per cent they constituted less than 25 per cent of the cells present.

4. An increase of protein was found in from 32 to 42 per cent of fluids. With the Noguchi test the fluid of 1 of 6 cases in the preparalytic stage yielded a positive reaction. The percentage of positives then became gradually higher to the third week after the onset of paralysis, when a rapid decrease in protein became apparent.

5. During the acute stages of poliomyelitis the fluids of 40 to 50 per cent of cases yielded a colloidal gold reaction of the luetic and meningitic zone types.

6. The potassium permanganate reduction test yielded positive reactions of indices over 2.3 with the spinal fluids of 41 per cent of cases examined from the second to twenty-first days after the onset of paralysis.

Every fluid was found to contain sufficient dextrose to reduce Fehling's solution in some degree.

8. Increased permeability of the meninges was indicated by the presence of natural antisheep hemolysis in the fluids of 66 per cent of cases in the acute stages and both the hemolysis and

[1] *Am. Journ. Med. Sci.* (1917), 144, 720–733.

a hemolytic complement in 30 per cent. Both of these substances were absent in the fluids of controls.

9. Substances inhibiting saponin hemolysis were not found in the fluids of cases, two to twenty-one days after the onset of paralysis.

10. A definite and absolute diagnostic criterion or laboratory test has not been discovered. A clear or slightly opalescent fluid flowing under increased pressure, sterile as examined by smear and culture when collected aseptically, poor in fibrin, reducing Fehling's solution, and containing an increased number of cells chiefly of the mononuclear variety are the most constant findings. An increase of protein and a high potassium permanganate reduction index strengthen the diagnosis, while a colloidal gold reaction of the luetic and meningitic zone types and the presence of natural antisheep hemolysis are helpful diagnostic data.

CRYPTOPLASMIC INFECTION

By Dr. H. W. Wade

The findings in cultures of material from a group of ulcers of undetermined causation seen in Hagonoy, Rizal, were described. Spirochætal, "blastomycotic," etc., etiology was negatived by absence of the characteristic microscopic findings, and yaws and syphilis could, it was thought, be eliminated.

In certain of the cultures of tissue fragments on special media the tissue cells, whether in spite of or because of overgrowth by bacteria contaminating the lesions, underwent apparently additive changes that sometimes produced deeply staining bodies not ordinarily seen in such material. These were referred to as "basic" bodies. In one culture from each of two cases, after considerable time, a *Cryptococcus* was found.

This fungus, classifiable as a *Cryptococcus*, was of an unusual type. It was not in the least saprophytic, did not even produce visible colonies, and did not multiply in subcultures. Therefore it could not be isolated.

The source of the fungus was not apparent. It was suggested that, in view of its late appearance in the cultures and its very unusual lack of cultivability, it might have arisen from the basic bodies. In this case it may have been concerned in the etiology of the lesions in an unrecognizable form.

These observations constitute a contribution toward the establishment of a hypothetical "cryptoplasmic" mode of infection. According to this hypothesis, which refers primarily to certain fungus infections, an invading organism may, whether occa-

sionally or regularly, wholly or in part, lose its morphologic, though retaining its biologic, identity and may exist and act in the form of a protoplasmic mass that, if it is not actually amorphous, is at least of such low morphological differentiation as not to be demonstrable. This substance is referred to as a "cryptoplasm." Other observations that have contributed to the formulation of this hypothesis are to be reported.

PROPHYLAXIS OF MALARIA

By Dr. Bernardo Samson

Doctor Samson outlined his methods of treating malaria both from the curative and the prophylactic viewpoints. He stated that one of the principal difficulties in connection with the prosecution of antimalarial campaigns in the provinces lay in the difficulty of establishing an understanding between the people and the health officers. The distribution of pamphlets printed in English, in Spanish, and in the vernacular he regarded as of doubtful utility. Better work, he thought, could be done by carefully arranged lectures, illustrated by lantern slides, given by the Bureau of Health officials or local physicians. Instruction in hygiene in the public schools, he believed, should include more information on mosquitoes and the symptoms and consequences of malaria. Doctor Samson said he thought much good would come by enlisting the coöperation of hacenderos employing large numbers of laborers on their plantations. He advocated the free distribution of quinine.—F. G. H.

R. B. Gibson,
Editor of the Proceedings,
Manila Medical Society.